Pentose phosphate pathway

The pentose phosphate pathway (PPP, also known as the *hexose monophosphate pathway)* is an oxidative metabolic pathway located in the cytoplasm, which, like glycolysis, starts from glucose 6-phosphate. It supplies two important precursors for anabolic pathways: **NADPH+H+,** which is required for the biosynthesis of fatty acids and isoprenoids, for example (see p. 168), and **ribose 5-phosphate,** a precursor in nucleotide biosynthesis (see p. 188).

A. Pentose phosphate pathway: oxidative part ◑

The **oxidative segment** of the PPP converts glucose 6-phosphate to ribulose 5-phosphate. One CO_2 and two NADPH+H^+ are formed in the process. Depending on the metabolic state, the much more complex **regenerative part** of the pathway (see **B**) can convert some of the pentose phosphates back to hexose phosphates, or it can pass them on to glycolysis for breakdown. In most cells, less than 10% of glucose 6-phosphate is degraded via the pentose phosphate pathway.

B. Reactions ◑

[1] The **oxidative part** starts with the oxidation of **glucose 6-phosphate** by *glucose-6-phosphate dehydrogenase*. This forms NADPH+H^+ for the first time. The second product, **6-phosphogluconolactone**, is an intramolecular ester (*lactone*) of 6-phosphogluconate.

[2] A specific hydrolase then cleaves the lactone, exposing the carboxyl group of **6-phosphogluconate**.

[3] The last enzyme in the oxidative part is *phosphogluconate dehydrogenase* [3], which releases the carboxylate group of 6-phosphogluconate as CO_2 and at the same time oxidizes the hydroxyl group at C_3 to an oxo group. In addition to a second NADPH+H^+, this also produces the ketopentose **ribulose 5-phosphate.** This is converted by an isomerase to ribose 5-phosphate, the initial compound for nucleotide synthesis (top).

The **regenerative part** of the PPP is only shown here schematically. A complete reaction scheme is given on p. 408. The function of the regenerative branch is to adjust the *net* production of NADPH+H^+ and pentose phosphates to the cell's current requirements. Normally, the demand for NADPH+H^+ is much higher than that for pentose phosphates. In these conditions, the reaction steps shown first convert six ribulose 5-phosphates to five molecules of fructose 6-phosphate and then, by isomerization, regenerate five glucose 6-phosphates. These can once again supply NADPH+H^+ to the oxidative part of the PPP. Repeating these reactions finally results in the oxidation of one glucose 6-phosphate into six CO_2. Twelve NADPH+H^+ arise in the same process. In sum, no pentose phosphates are produced via this pathway.

In the recombination of sugar phosphates in the regenerative part of the PPP, there are two enzymes that are particularly important:

[5] *Transaldolase* transfers C_3 units from sedoheptulose 7-phosphate, a ketose with seven C atoms, to the aldehyde group of glyceraldehyde 3-phosphate.

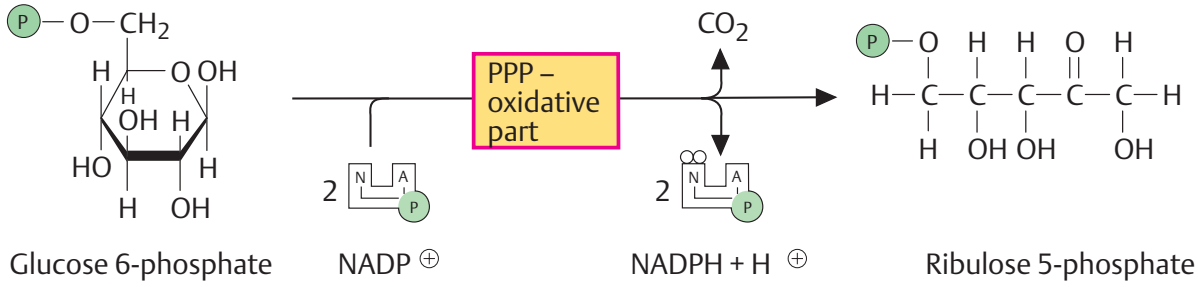
[4] *Transketolase,* which contains thiamine diphosphate, transfers C_2 fragments from one sugar phosphate to another.

The reactions in the regenerative segment of the PPP are freely reversible. It is therefore easily possible to use the regenerative part of the pathway to convert hexose phosphates into pentose phosphates. This can occur when there is a high demand for pentose phosphates—e.g., during DNA replication in the S phase of the cell cycle (see p. 394).

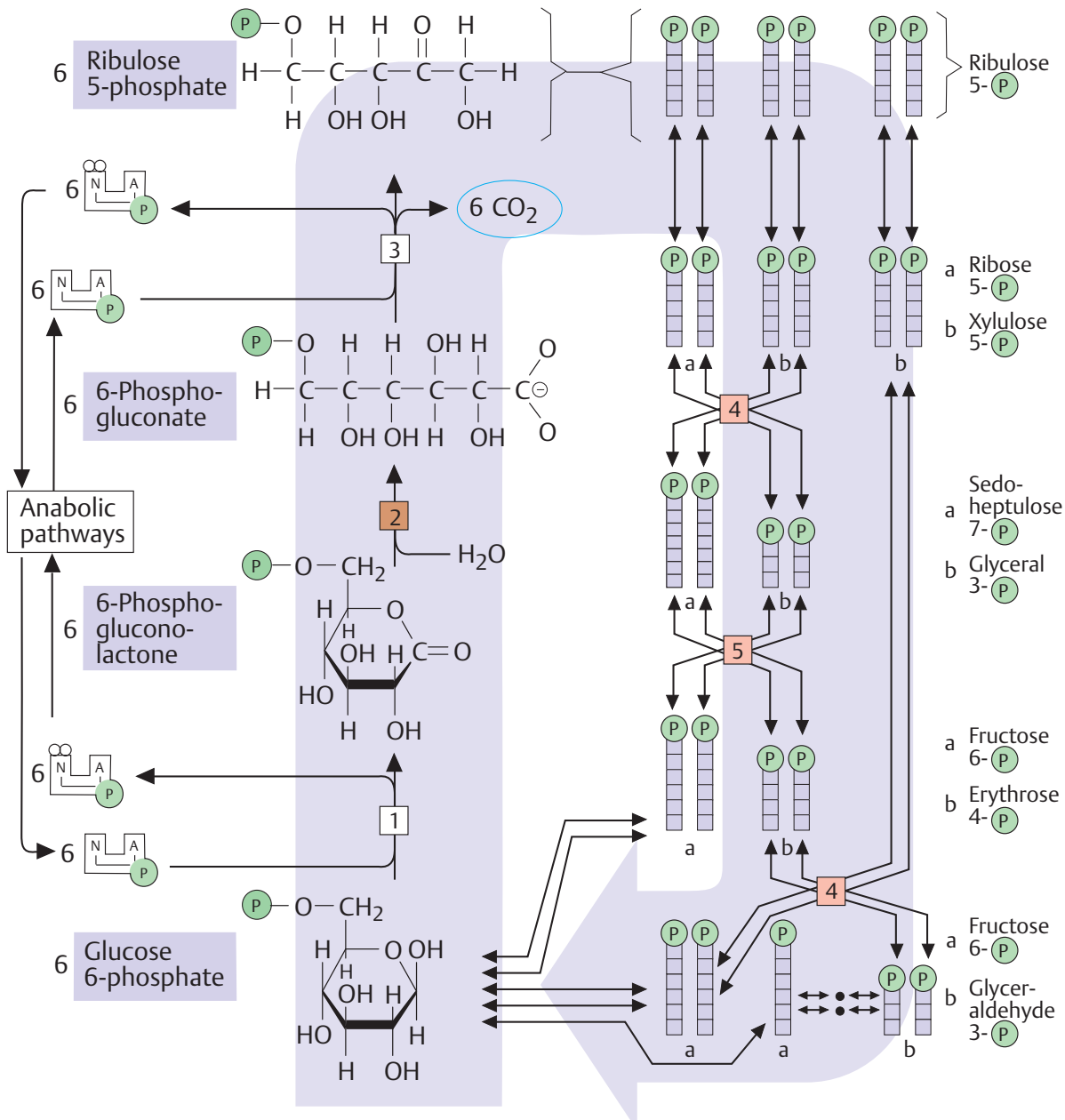
Additional information

When energy in the form of ATP is required in addition to NADPH+H^+, the cell is able to channel the products of the regenerative part of the PPP (fructose 6-phosphate and glyceraldehyde 3-phosphate) into glycolysis. Further degradation is carried out via the tricarboxylic acid cycle and the respiratory chain to CO_2 and water. Overall, the cell in this way obtains 12 mol NADPH+H^+ and around 150 mol ATP from 6 mol glucose 6-phosphate. PPP activity is stimulated by *insulin* (see p. 388). This not only increases the rate of glucose degradation, but also produces additional NADPH+H^+ for fatty acid synthesis (see p. 168).

A. Pentose phosphate pathway: oxidative part



B. Reactions



1 Glucose 6-phosphate dehydrogenase *1.1.1.49*

2 Gluconolactonase *3.1.1.17*

3 Phosphogluconate dehydrogenase (decarboxylating) *1.1.1.44*

4 Transketolase *2.2.1.1*

5 Transaldolase *2.2.1.2*

Gluconeogenesis

Some tissues, such as *brain* and *erythrocytes*, depend on a constant supply of glucose. If the amount of carbohydrate taken up in food is not suf cient, the blood sugar level can be maintained for a limited time by *degradation of hepatic glycogen* (see p. 156). If these reserves are also exhausted, de-novo synthesis of glucose (**gluconeogenesis**) begins. The **liver** is also mainly responsible for this (see p. 310), but the tubular cells of the **kidney** also show a high level of gluconeogenetic activity (see p. 328). The main precursors for gluconeogenesis are **amino acids** derived from muscle proteins. Another important precursor is **lactate,** which is formed in erythrocytes and muscle proteins when there is oxygen deficiency. **Glycerol** produced from the degradation of fats can also be used for gluconeogenesis. However, the conversion of fatty acids into glucose is *not* possible in animal metabolism (see p. 138). The human organism can synthesize several hundred grams of glucose per day by gluconeogenesis.

A. Gluconeogenesis ◑

Many of the reaction steps involved in gluconeogenesis are catalyzed by the same enzymes that are used in glycolysis (see p. 150). Other enzymes are specific to gluconeogenesis and are only synthesized, under the influence of *cortisol* and *glucagon* when needed (see p. 158). Glycolysis takes place exclusively when needed in the cytoplasm, but gluconeogenesis also involves the *mitochondria* and the *endoplasmic reticulum* (ER). Gluconeogenesis consumes 4 ATP (3 ATP + 1 GTP) per glucose—i. e., twice as many as glycolysis produces.

[1] **Lactate** as a precursor for gluconeogenesis is mainly derived from muscle (see Cori cycle, p. 338) and erythrocytes. LDH (see p. 98) oxidizes lactate to pyruvate, with $NADH+H^+$ formation.

[2] The first steps of actual gluconeogenesis take place in the *mitochondria.* The reason for this "detour" is the equilibrium state of the pyruvate kinase reaction (see p. 150). Even coupling to ATP hydrolysis would not be sufficient to convert pyruvate *directly* into phospho*enol* pyruvate (PEP). **Pyruvate** derived from lactate or amino acids is therefore initially transported into the mitochondrial matrix, and—in a biotin-dependent reaction catalyzed by *pyruvate carboxylase*—is carboxylated there to **oxaloacetate**. Oxaloacetate is also an intermediate in the tricarboxylic acid cycle. *Amino acids* with breakdown products that enter the cycle or supply pyruvate can therefore be converted into glucose (see p. 180).

[3] The oxaloacetate formed in the mitochondrial matrix is initially reduced to **malate**, which can leave the mitochondria via inner membrane transport systems (see p. 212).

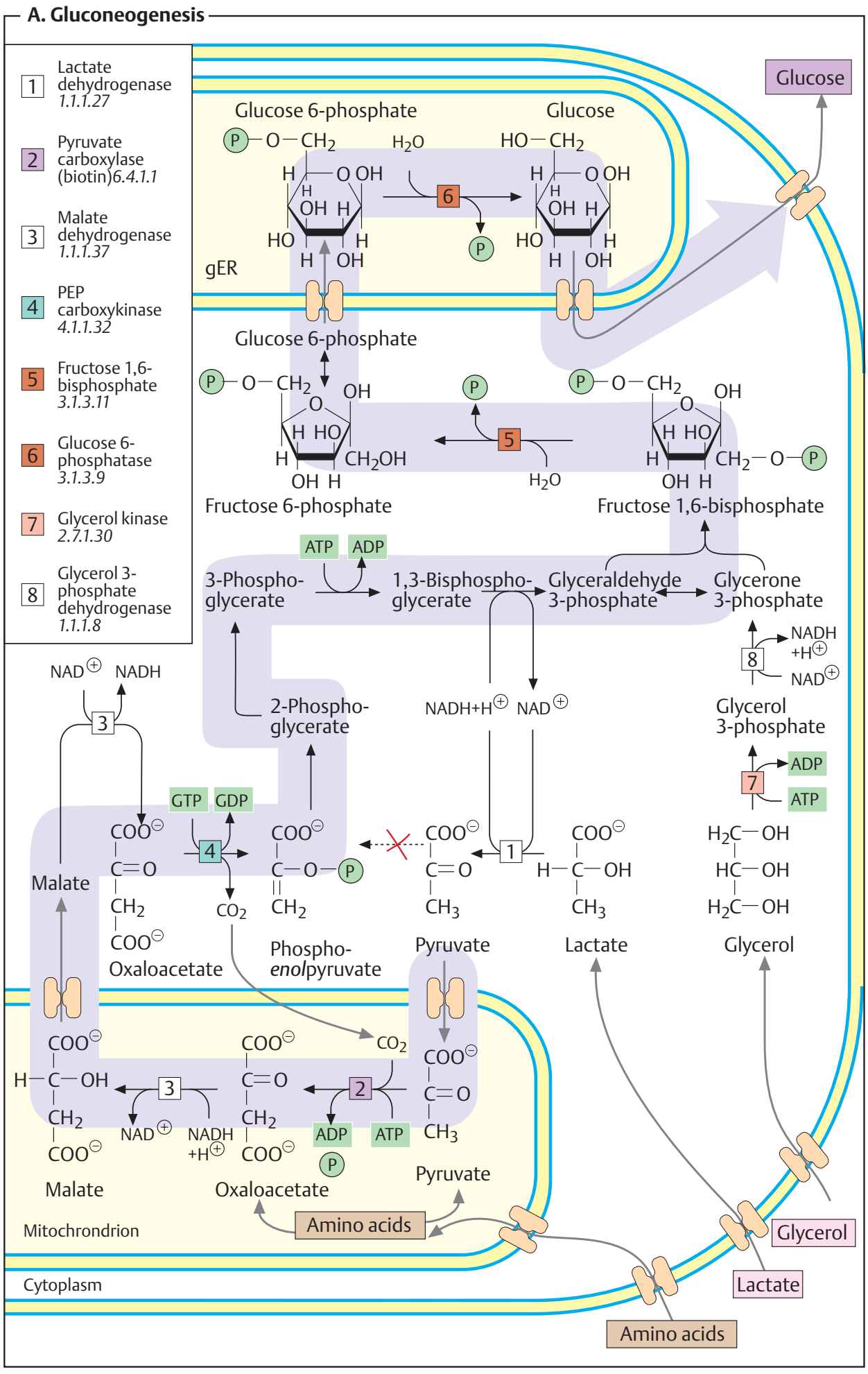
[4] In the cytoplasm, oxaloacetate is reformed and then converted into **phosphoenol pyruvate** by a GTP-dependent *PEP carboxykinase.* The subsequent steps up to fructose 1,6-bisphosphate represent the reverse of the corresponding reactions involved in glycolysis. One additional ATP per C_3 fragment is used for the synthesis of 1,3-bisphosphoglycerate.

Two gluconeogenesis-specific phosphatases then successively cleave off the phosphate residues from **fructose 1,6-bisphosphate**. In between these reactions lies the isomerization of fructose 6-phosphate to **glucose 6-phosphate**—another glycolytic reaction.

[5] The reaction catalyzed by *fructose 1,6-bisphosphatase* is an important regulation point in gluconeogenesis (see p. 158).

[6] The last enzyme in the pathway, *glucose 6-phosphatase,* occurs in the liver, but not in muscle. It is located in the interior of the smooth endoplasmic reticulum. Specific transporters allow glucose 6-phosphate to enter the ER and allow the **glucose** formed there to return to the cytoplasm. From there, it is ultimately released into the blood.

Glycerol initially undergoes phosphorylation at C-3 [7]. The **glycerol 3-phosphate** formed is then oxidized by an NAD^+-dependent dehydrogenase to form **glycerone 3-phosphate** [8] and thereby channeled into gluconeogenesis. An FAD-dependent mitochondrial enzyme is also able to catalyze this reaction (known as the "glycerophosphate shuttle"; see p. 212).



Glycogen metabolism

Glycogen (see p. 40) is used in animals as a **carbohydrate reserve,** from which glucose phosphates and glucose can be released when needed. Glucose storage itself would not be useful, as high concentrations within cells would make them strongly hypertonic and would therefore cause an influx of water. By contrast, insoluble glycogen has only low osmotic activity.

A. Glycogen balance ◑

Animal glycogen, like amylopectin in plants, is a *branched homopolymer of glucose.* The glucose residues are linked by an α1→4-glycosidic bond. Every tenth or so glucose residue has an additional α1→6 bond to another glucose. These branches are extended by additional α1→4-linked glucose residues. This structure produces tree-shaped molecules consisting of up to 50000 residues ($M > 1 \quad 10^7$ Da).

Hepatic glycogen is never completely degraded. In general, only the nonreducing ends of the "tree" are shortened, or—when glucose is abundant—elongated. The reducing end of the tree is linked to a special protein, **glycogenin**. Glycogenin carries out autocatalytic covalent bonding of the first glucose at one of its tyrosine residues and elongation of this by up to seven additional glucose residues. It is only at this point that *glycogen synthase* becomes active to supply further elongation.

[1] The formation of glycosidic bonds between sugars is *endergonic.* Initially, therefore, the activated form—**UDP-glucose**—is synthesized by reaction of glucose 1-phosphate with UTP (see p. 110).

[2] *Glycogen synthase* now transfers glucose residues one by one from UDP-glucose to the non-reducing ends of the available "branches."

[3] Once the growing chain has reached a specific length (> 11 residues), the *branching enzyme* cleaves an oligosaccharide consisting of 6–7 residues from the end of it, and adds this into the interior of the same chain or a neighboring one with α1→6 linkage. These **branches** are then further extended by glycogen synthase.

[4] The branched structure of glycogen allows rapid release of sugar residues. The most important degradative enzyme, *glycogen phosphorylase,* cleaves residues from a nonreducing end one after another as **glucose 1-phosphate**. The larger the number of these ends, the more phosphorylase molecules can attack simultaneously. The formation of glucose 1-phosphate instead of glucose has the advantage that no ATP is needed to channel the released residues into glycolysis or the PPP.

[5] [6] Due to the structure of glycogen phosphorylase, degradation comes to a halt four residues away from each branching point. Two more enzymes overcome this blockage. First, a *glucanotransferase* moves a trisaccharide from the side chain to the end of the main chain [5]. A *1,6-glucosidase* [6] then cleaves the single remaining residue as a free glucose and leaves behind an unbranched chain that is once again accessible to phosphorylase.

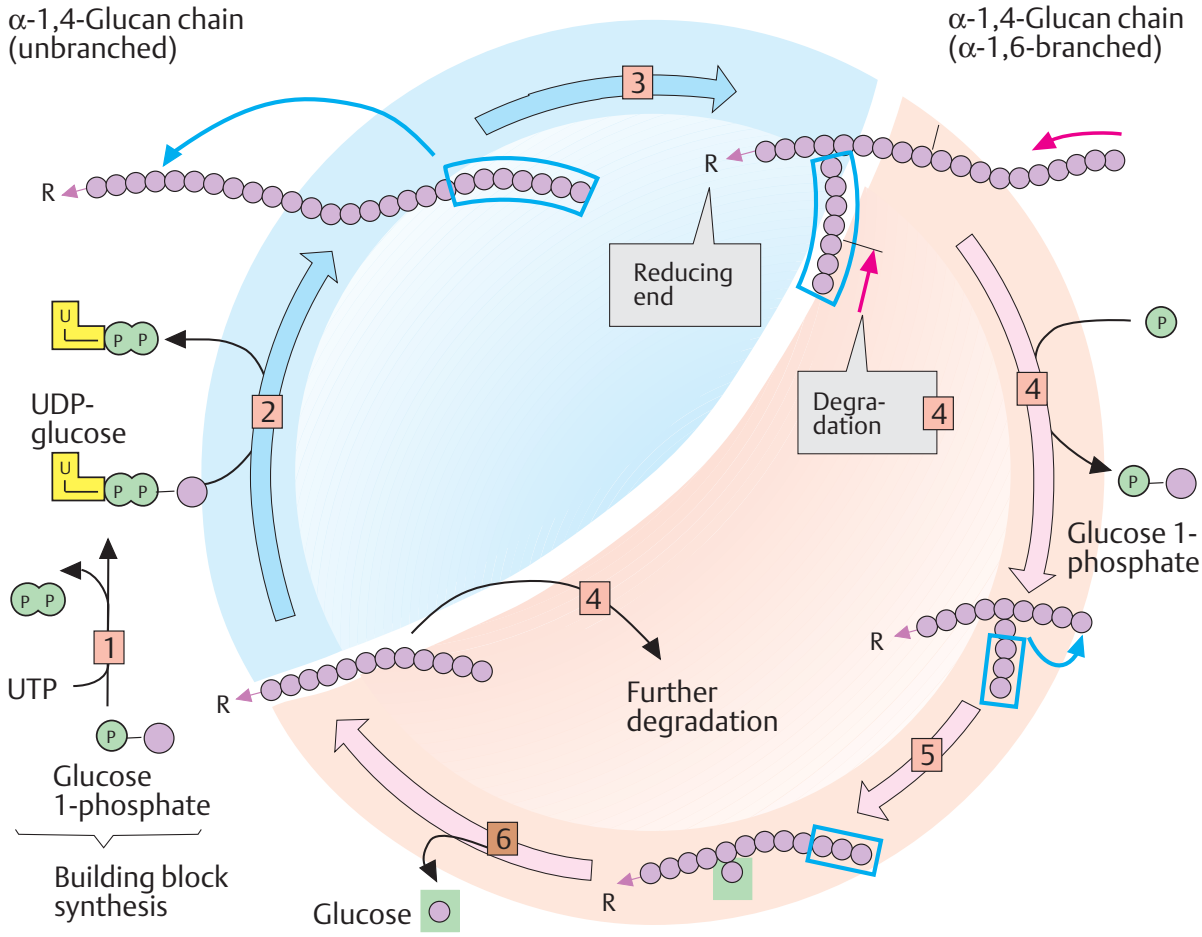
The **regulation of glycogen metabolism** by interconversion, and the role of hormones in these processes, are discussed on p. 120.

B. Glycogen balance ◑

The human organism can store up to 450 g of glycogen—one-third in the **liver** and almost all of the remainder in **muscle.** The glycogen content of the other organs is low.

Hepatic glycogen is mainly used to maintain the *blood glucose level* in the postresorptive phase (see p. 308). The glycogen content of the liver therefore varies widely, and can decline to almost zero in periods of extended hunger. After this, gluconeogenesis (see p. 154) takes over the glucose supply for the organism. *Muscle glycogen* serves as an *energy reserve* and is not involved in blood glucose regulation. Muscle does not contain any glucose 6-phosphatase and is therefore unable to release glucose into the blood. The glycogen content of muscle therefore does not fluctuate as widely as that of the liver.

A. Glycogen metabolism



1 UTP-glucose 1-phosphate uridyltransferase *2.7.7.9*

2 Glycogen synthase *2.4.1.11*

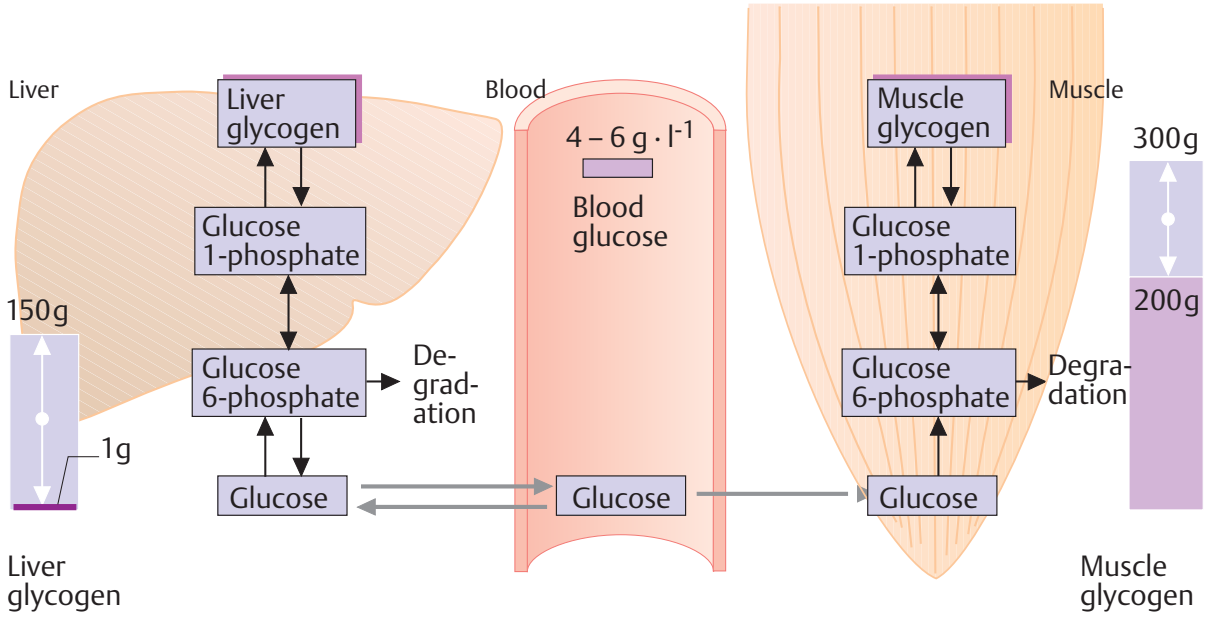
3 Glucan branching enzyme *2.4.1.18*

4 Phosphorylase *2.4.1.1*

5 4-α-Glucanotransferase *2.4.1.25*

6 Amylo-1,6-glucosidase *3.2.1.33*

B. Glycogen balance



Regulation

A. Regulation of carbohydrate metabolism ◑

In all organisms, carbohydrate metabolism is subject to complex regulatory mechanisms involving *hormones, metabolites*, and *coenzymes*. The scheme shown here (still a simplified one) applies to the liver, which has central functions in carbohydrate metabolism (see p. 306). Some of the control mechanisms shown here are not effective in other tissues.

One of the liver's most important tasks is to store excess glucose in the form of glycogen and to release glucose from glycogen when required *(buffer function)*. When the glycogen reserves are exhausted, the liver can provide glucose by de novo synthesis (*gluconeogenesis*; see p. 154). In addition, like all tissues, the liver breaks glucose down via glycolysis. These functions have to be coordinated with each other. For example, there is no point in glycolysis and gluconeogenesis taking place *simultaneously*, and glycogen synthesis and glycogen degradation should not occur simultaneously either. This is ensured by the fact that two *different* enzymes exist for important steps in both pathways, each of which catalyzes only the anabolic or the catabolic reaction. The enzymes are also regulated differently. Only these key enzymes are shown here.

Hormones. The hormones that influence carbohydrate metabolism include the peptides insulin and glucagon; a glucocorticoid, cortisol; and a catecholamine, epinephrine (see p. 380). **Insulin** activates *glycogen synthase* ([1]; see p. 388), and induces several enzymes involved in glycolysis [3, 5, 7]. At the same time, insulin inhibits the synthesis of enzymes involved in gluconeogenesis (*repression;* [4, 6, 8, 9]). **Glucagon,** the antagonist of insulin, has the opposite effect. It induces gluconeogenesis enzymes [4, 6, 8, 9] and represses *pyruvate kinase* [7], a key enzyme of glycolysis. Additional effects of glucagon are based on the *interconversion* of enzymes and are mediated by the second messenger cAMP. This inhibits glycogen synthesis [1] and activates glycogenolysis [2]. Epinephrine acts in a similar fashion. The inhibition of *pyruvate kinase* [7] by glucagon is also due to interconversion.

Glucocorticoids—mainly **cortisol** (see p. 374)—induce all of the key enzymes involved in gluconeogenesis [4, 6, 8, 9]. At the same time, they also induce enzymes involved in amino acid degradation and thereby provide precursors for gluconeogenesis. Regulation of the expression of *PEP carboxykinase,* a key enzyme in gluconeogenesis, is discussed in detail on p. 244.

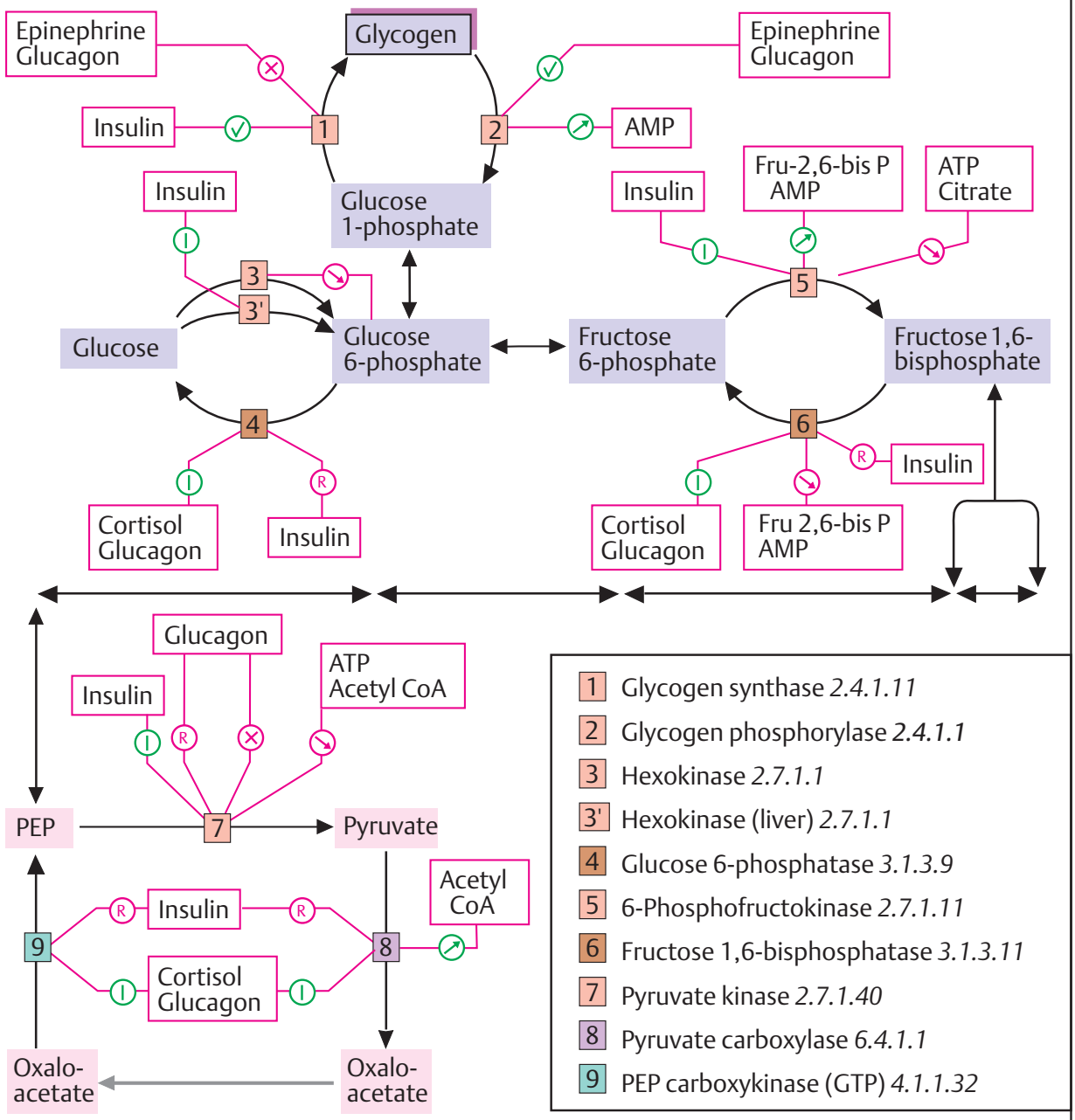
Metabolites. High concentrations of **ATP** and **citrate** inhibit glycolysis by allosteric regulation of *phosphofructokinase*. ATP also inhibits *pyruvate kinase*. **Acetyl-CoA,** an inhibitor of *pyruvate kinase,* has a similar effect. All of these metabolites arise from glucose degradation *(feedback inhibition)*. **AMP** and **ADP**, signals for ATP deficiency, activate glycogen degradation and inhibit gluconeogenesis.

B. Fructose 2,6-bisphosphate ◑

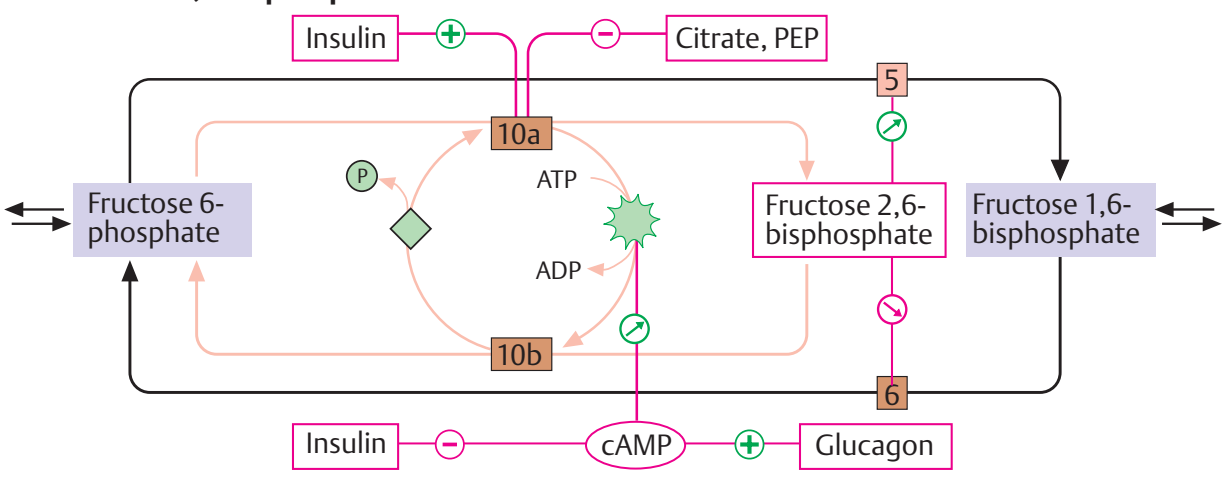
Fructose 2,6-bisphosphate (Fru-2,6-bP) plays an important part in carbohydrate metabolism. This metabolite is formed in small quantities from fructose 6-phosphate and has purely *regulatory functions*. It stimulates glycolysis by allosteric activation of *phosphofructokinase* and inhibits gluconeogenesis by inhibition of *fructose 1,6-bisphosphatase*.

The synthesis and degradation of Fru-2,6-bP are catalyzed by one and the same protein [10a, 10b]. If the enzyme is present in an unphosphorylated form [10a], it acts as a kinase and leads to the formation of Fru-2,6-bP. After phosphorylation by cAMP-dependent protein kinase A (PK-A), it acts as a phosphatase [10b] and now catalyzes the degradation of Fru-2,6-bP to fructose 6-phosphate. The equilibrium between [10a] and [10b] is regulated by hormones. Epinephrine and glucagon increase the cAMP level (see p. 120). As a result of increased PK-A activity, this reduces the Fru-2,6-bP concentration and inhibits glycolysis, while at the same time activating gluconeogenesis. Conversely, via [10a], insulin activates the synthesis of Fru-2,6-bP and thus glycolysis. In addition, insulin also inhibits the action of glucagon by reducing the cAMP level (see p. 120).

A. Regulation of carbohydrate metabolism



B. Fructose 2,6-bisphosphate



Diabetes mellitus

Diabetes mellitus is a very common metabolic disease that is caused by absolute or relative insulin deficiency. The lack of this peptide hormone (see p. 76) mainly affects carbohydrate and lipid metabolism. Diabetes mellitus occurs in two forms. In **type 1** diabetes (insulin-dependent diabetes mellitus, IDDM), the insulin-forming cells are destroyed in young individuals by an autoimmune reaction. The less severe **type 2** diabetes (non-insulin-dependent diabetes mellitus, NIDDM) usually has its first onset in elderly individuals. The causes have not yet been explained in detail in this type.

A. Insulin biosynthesis ○

Insulin is produced by the B cells of the *islets of Langerhans* in the pancreas. As is usual with secretory proteins, the hormone's precursor (*preproinsulin*) carries a signal peptide that directs the peptide chain to the interior of the endoplasmic reticulum (see p. 210). *Proinsulin* is produced in the ER by cleavage of the signal peptide and formation of disulfide bonds. Proinsulin passes to the Golgi apparatus, where it is packed into vesicles—the β-*granules.* After cleavage of the *C peptide*, *mature insulin* is formed in the β-granules and is stored in the form of zinc-containing hexamers until secretion.

B. Effects of insulin deficiency ◑

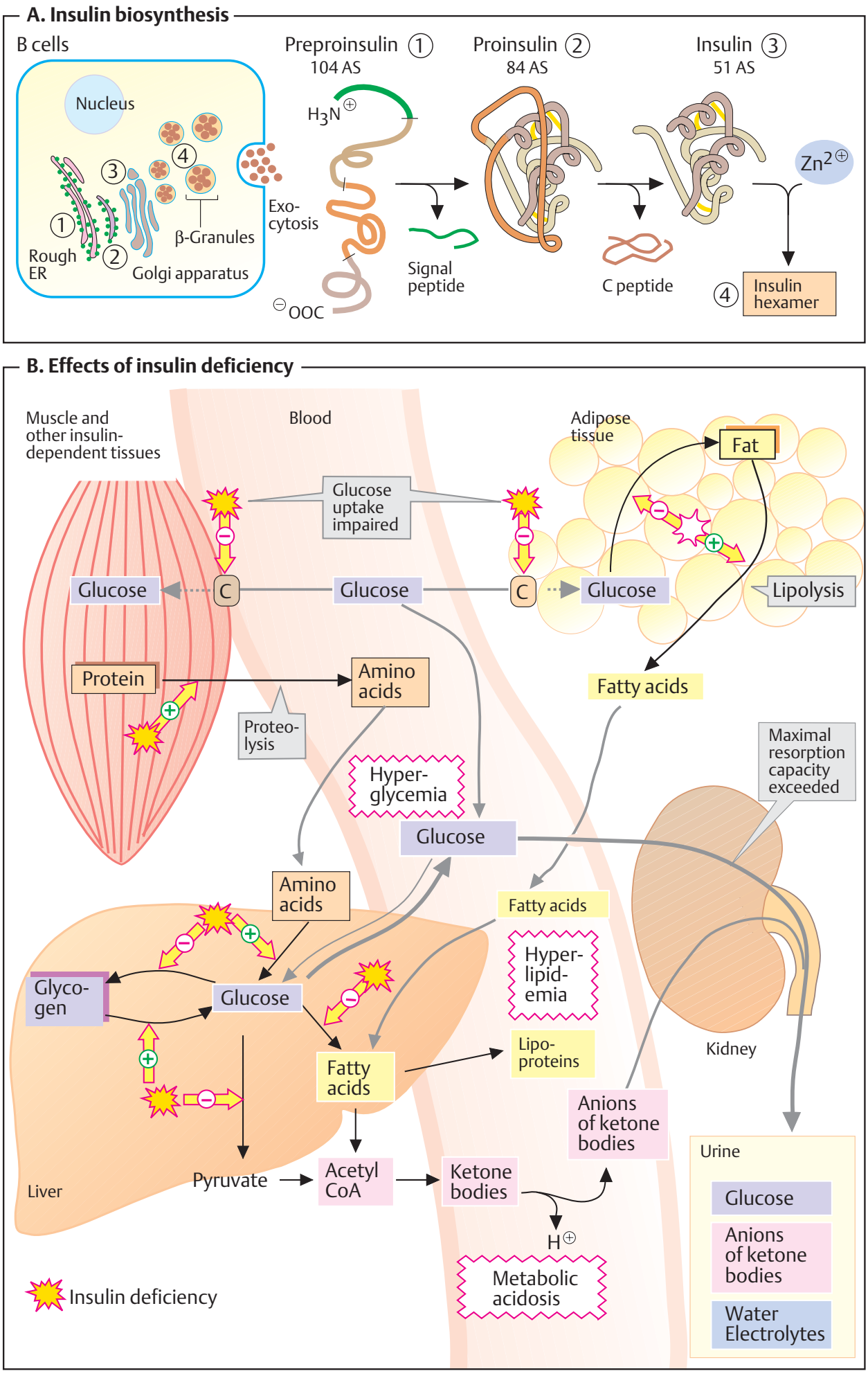
The effects of insulin on **carbohydrate metabolism** are discussed on p. 158. In simplified terms, they can be described as *stimulation of glucose utilization* and *inhibition of gluconeogenesis*. In addition, the transport of glucose from the blood into most tissues is also insulin-dependent (exceptions to this include the liver, CNS, and erythrocytes).

The **lipid metabolism** of adipose tissue is also influenced by the hormone. In these cells, insulin stimulates the reorganization of glucose into fatty acids. This is mainly based on activation of *acetyl CoA carboxylase* (see p. 162) and increased availability of $NADPH+H^+$ due to increased PPP activity (see p. 152). On the other hand, insulin also inhibits the degradation of fat by hormone-sensitive lipases (see p. 162) and prevents the breakdown of muscle protein.

The effects of insulin *deficiency* on metabolism are shown by arrows in the illustration. Particularly noticeable is the increase in the glucose concentration in the blood, from 5 mM to 9 mM (90 mg dL^{-1}) or more (**hyperglycemia,** elevated blood glucose level). In *muscle* and *adipose tissue* – the two most important glucose consumers—glucose uptake and glucose utilization are impaired by insulin deficiency. Glucose utilization in the *liver* is also reduced. At the same time, gluconeogenesis is stimulated, partly due to increased proteolysis in the muscles. This increases the blood sugar level still further. When the capacity of the *kidneys* to resorb glucose is exceeded (at plasma concentrations of 9 mM or more), glucose is excreted in the urine (**glucosuria**).

The increased degradation of fat that occurs in insulin deficiency also has serious effects. Some of the fatty acids that accumulate in large quantities are taken up by the liver and used for lipoprotein synthesis **(hyperlipidemia)**, and the rest are broken down into acetyl CoA. As the tricarboxylic acid cycle is not capable of taking up such large quantities of acetyl CoA, the excess is used to form **ketone bodies** (*acetoacetate* and *β-hydroxybutyrate* see p. 312). As H^+ ions are released in this process, diabetics not receiving adequate treatment can suffer severe **metabolic acidosis** (diabetic coma). The *acetone* that is also formed gives these patients' breath a characteristic odor. In addition, large amounts of ketone body anions appear in the urine (**ketonuria**).

Diabetes mellitus can have serious secondary effects. A constantly raised blood sugar level can lead in the long term to changes in the blood vessels (diabetic angiopathy), kidney damage (nephropathy) and damage to the nervous system (neuropathy), as well as to cataracts in the eyes.



Overview

A. Fat metabolism ◑

Fat metabolism in adipose tissue (top). Fats (triacylglycerols) are the most important energy reserve in the animal organism. They are mostly stored in insoluble form in the cells of adipose tissue—the *adipocytes*—where they are constantly being synthesized and broken down again.

As precursors for the biosynthesis of fats (**lipogenesis**), the adipocytes use triacylglycerols from lipoproteins (VLDLs and chylomicrons; see p. 278), which are formed in the liver and intestines and delivered by the blood. *Lipoprotein lipase* [1], which is located on the inner surface of the blood capillaries, cleaves these triacylglycerols into glycerol and fatty acids, which are taken up by the adipocytes and converted back into fats.

The degradation of fats (**lipolysis**) is catalyzed in adipocytes by *hormone-sensitive lipase* [2]—an enzyme that is regulated by various hormones by cAMP-dependent *interconversion* (see p. 120). The amount of fatty acids released depends on the activity of this lipase; in this way, the enzyme regulates the plasma levels of fatty acids.

In the blood plasma, fatty acids are transported in free form—i. e., non-esterified. Only short-chain fatty acids are soluble in the blood; longer, less water-soluble fatty acids are transported bound to albumin.

Degradation of fatty acids in the liver (left). Many tissues take up fatty acids from the blood plasma in order to synthesize fats or to obtain energy by oxidizing them. The metabolism of fatty acids is particularly intensive in the hepatocytes in the liver.

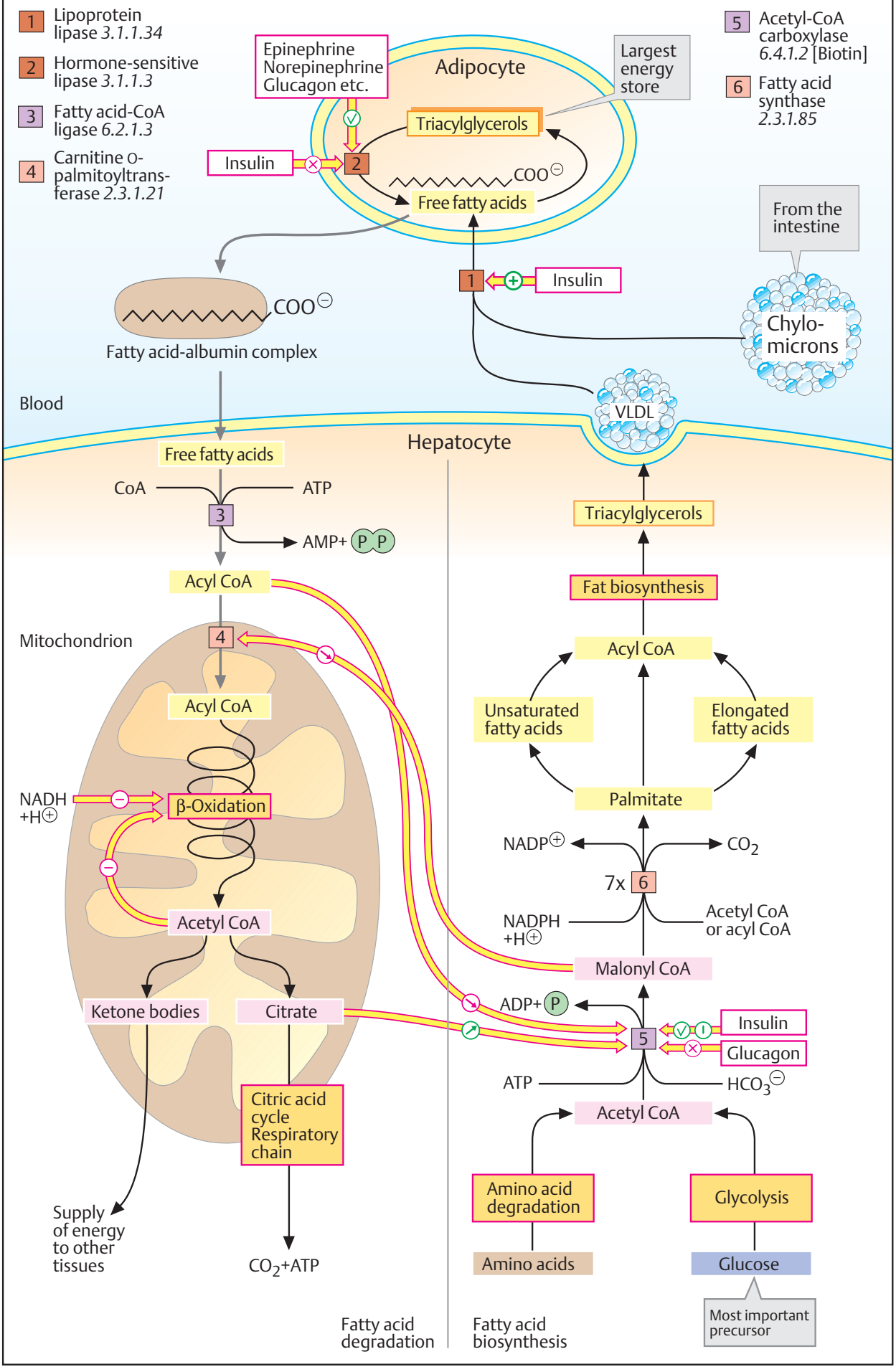
The most important process in the degradation of fatty acids is **β-oxidation**—a metabolic pathway in the mitochondrial matrix (see p. 164). Initially, the fatty acids in the cytoplasm are activated by binding to coenzyme A into **acyl CoA** [3]. Then, with the help of a transport system (the carnitine shuttle [4]; see p. 164), the activated fatty acids enter the mitochondrial matrix, where they are broken down into **acetyl CoA**. The resulting acetyl residues can be oxidized to CO_2 in the tricarboxylic acid cycle, producing reduced coenzyme and ATP derived from it by oxidative phosphorylation. If acetyl CoA production exceeds the energy requirements of the hepatocytes—as is the case when there is a high level of fatty acids in the blood plasma (typical in hunger and diabetes mellitus)—then the excess is converted into **ketone bodies** (see p. 312). These serve exclusively to supply other tissues with energy.

Fat synthesis in the liver (right). Fatty acids and fats are mainly synthesized in the liver and in adipose tissue, as well as in the kidneys, lungs, and mammary glands. Fatty acid biosynthesis occurs in the cytoplasm—in contrast to fatty acid degradation. The most important precursor is **glucose**, but certain amino acids can also be used.

The first step is carboxylation of **acetyl CoA** to **malonyl CoA**. This reaction is catalyzed by *acetyl-CoA carboxylase* [5], which is the *key enzyme in fatty acid biosynthesis.* Synthesis into fatty acids is carried out by *fatty acid synthase* [6]. This multifunctional enzyme (see p. 168) starts with one molecule of acetyl-CoA and elongates it by adding malonyl groups in seven reaction cycles until palmitate is reached. One CO_2 molecule is released in each reaction cycle. The fatty acid therefore grows by two carbon units each time. $NADPH+H^+$ is used as the reducing agent and is derived either from the *pentose phosphate pathway* (see p. 152) or from *isocitrate dehydrogenase* and *malic enzyme* reactions.

The elongation of the fatty acid by *fatty acid synthase* concludes at C_{16}, and the product, **palmitate** (16:0), is released. Unsaturated fatty acids and long-chain fatty acids can arise from palmitate in subsequent reactions. Fats are finally synthesized from activated fatty acids (acyl CoA) and glycerol 3-phosphate (see p. 170). To supply peripheral tissues, fats are packed by the hepatocytes into lipoprotein complexes of the VLDL type and released into the blood in this form (see p. 278).

A. Fat metabolism



Fatty acid degradation

A. Fatty acid degradation: β-oxidation ◑

After uptake by the cell, fatty acids are activated by conversion into their CoA derivatives—**acyl CoA** is formed. This uses up two energy-rich anhydride bonds of ATP per fatty acid (see p. 162). For channeling into the mitochondria, the acyl residues are first transferred to *carnitine* and then transported across the inner membrane as **acyl carnitine** (see **B**).

The degradation of the fatty acids occurs in the mitochondrial matrix through an oxidative cycle in which C_2 units are successively cleaved off as **acetyl CoA** (*activated acetic acid*). Before the release of the acetyl groups, each CH_2 group at C-3 of the acyl residue (the β-C atom) is oxidized to the keto group—hence the term **β-oxidation** for this metabolic pathway. Both spatially and functionally, it is closely linked to the tricarboxylic acid cycle (see p. 136) and to the respiratory chain (see p. 140).

[1] The first step is dehydrogenation of **acyl CoA** at C-2 and C-3. This yields an unsaturated Δ^2-enoyl-CoA derivative with a *trans*-configured double bond. The two hydrogen atoms are initially transferred from FAD-containing *acyl CoA dehydrogenase* to the **electron-transferring flavoprotein (ETF)**. *ETF dehydrogenase* [5] passes them on from ETF to ubiquinone (coenzyme Q), a component of the *respiratory chain* (see p. 140). Other FAD-containing mitochondrial dehydrogenases are also able to supply the respiratory chain with electrons in this fashion.

There are three *isoenzymes* (see p. 98) of *acyl CoA dehydrogenase* that are specialized for long-chain fatty acids (12–18 C atoms), medium-chain fatty acids (4–14), and short-chain fatty acids (4–8).

[2] The next step in fatty acid degradation is the addition of a water molecule to the double bond of the enoyl CoA (*hydration*), with formation of **β-hydroxyacyl CoA**.

[3] In the next reaction, the OH group at C-3 is oxidized to a carbonyl group (*dehydrogenation*). This gives rise to **β-ketoacyl CoA**, and the reduction equivalents are transferred to NAD^+, which also passes them on to the *respiratory chain.*

[4] β-Ketoacyl-CoA is now broken down by an *acyl transferase* into **acetyl CoA** and an **acyl CoA shortened by 2 C atoms** (*"thioclastic cleavage"*).

Several cycles are required for complete degradation of long-chain fatty acids—eight cycles in the case of stearyl-CoA (C18:0), for example. The acetyl CoA formed can then undergo further metabolism in the *tricarboxylic acid cycle* (see p. 136), or can be used for biosynthesis. When there is an excess of acetyl CoA, the liver can also form ketone bodies (see p. 312).

When oxidative degradation is complete, one molecule of palmitic acid supplies around 106 molecules of ATP, corresponding to an energy of 3300 kJ mol^{-1}. This high energy yield makes fats an ideal form of storage for metabolic energy. Hibernating animals such as polar bears can meet their own energy requirements for up to 6 months solely by fat degradation, while at the same time producing the vital water they need via the respiratory chain ("respiratory water").

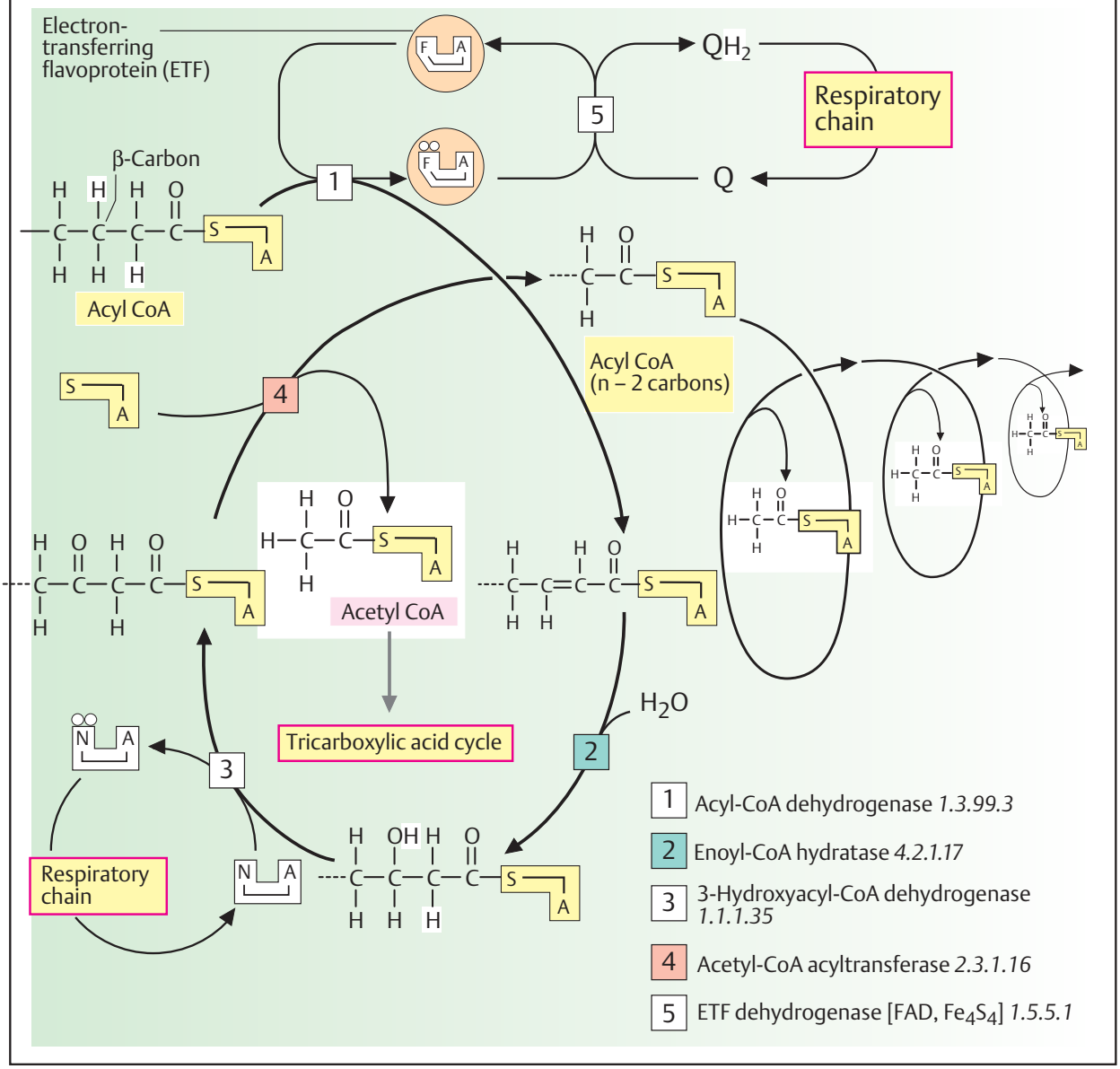
B. Fatty acid transport ◑

The inner mitochondrial membrane has a group-specific transport system for fatty acids. In the cytoplasm, the acyl groups of activated fatty acids are transferred to **carnitine** by *carnitine acyltransferase* [1]. They are then channeled into the matrix by an acylcarnitine/carnitine antiport as **acyl carnitine**, in exchange for free carnitine. In the matrix, the mitochondrial enzyme *carnitine acyltransferase* catalyzes the return transfer of the acyl residue to CoA.

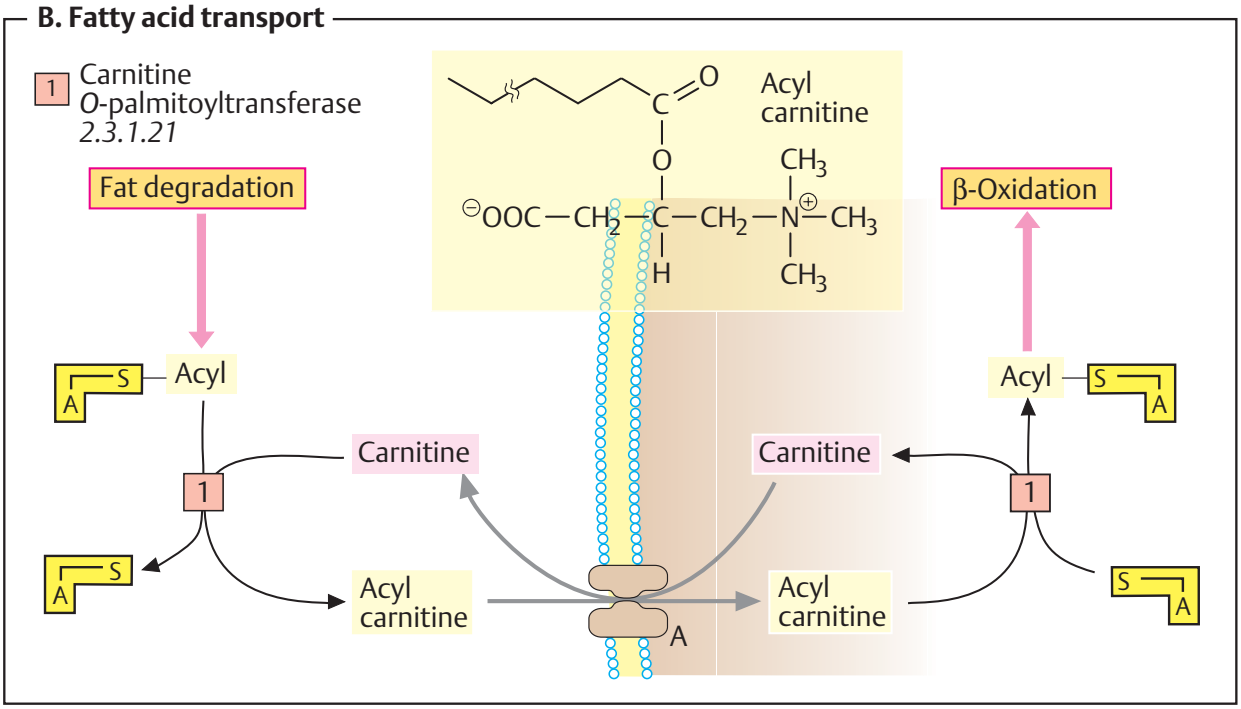
The carnitine shuttle is the rate-determining step in mitochondrial fatty acid degradation. Malonyl CoA, a precursor of fatty acid biosynthesis, inhibits *carnitine acyltransferase* (see p. 162), and therefore also inhibits uptake of fatty acids into the mitochondrial matrix.

The most important regulator of β-oxidation is the $NAD^+/NADH+H^+$ ratio. If the respiratory chain is not using any $NADH+H^+$, then not only the tricarboxylic acid cycle (see p. 136) but also β-oxidation come to a standstill due to the lack of NAD^+.

A. Fatty acid degradation: β-oxidation



B. Fatty acid transport



Minor pathways of fatty acid degradation

Most fatty acids are saturated and even-numbered. They are broken down via **β-oxidation** (see p.164). In addition, there are special pathways involving degradation of unsaturated fatty acids (**A**), degradation of fatty acids with an odd number of C atoms (**B**), α and ω oxidation of fatty acids, and degradation in peroxisomes.

A. Degradation of unsaturated fatty acids ○

Unsaturated fatty acids usually contain a *cis* double bond at position 9 or 12—e. g., linoleic acid (18:2; 9,12). As with saturated fatty acids, degradation in this case occurs via β-oxidation until the C-9-*cis* double bond is reached. Since *enoyl-CoA hydratase* only accepts substrates with *trans* double bonds, the corresponding enoyl-CoA is converted by an isomerase from the *cis*-Δ^3, *cis*- Δ^6 isomer into the *trans*-Δ^3,*cis*-Δ^6 isomer [1]. Degradation by β-oxidation can now continue until a shortened *trans*-Δ^2, *cis*-Δ^4 derivative occurs in the next cycle. This cannot be isomerized in the same way as before, and instead is reduced in an NADPH-dependent way to the *trans*-Δ^3 compound [2]. After rearrangement by *enoyl-CoA isomerase* [1], degradation can finally be completed via normal β-oxidation.

B. Degradation of oddnumbered fatty acids ○

Fatty acids with an odd number of C atoms are treated in the same way as "normal" fatty acids—i. e., they are taken up by the cell with ATP-dependent activation to acyl CoA and are transported into the mitochondria with the help of the carnitine shuttle and broken down there by β–oxidation (see p. 164). In the last step, **propionyl CoA** arises instead of acetyl CoA. This is first carboxylated by *propionyl CoA carboxylase* into ***(S)*-methylmalonyl CoA** [3], which—after isomerization into the (*R*) enantiomer (not shown; see p. 411)—is isomerized into **succinyl CoA** [4].

Various coenzymes are involved in these reactions. The carboxylase [3] requires *biotin,* and the mutase [4] is dependent on *coenzyme* B_{12} (5′-deoxyadenosyl cobalamin; see p. 108). Succinyl-CoA is an intermediate in the tricarboxylic acid cycle and is available for *gluconeogenesis* through conversion into oxaloacetate. Odd-numbered fatty acids from propionyl-CoA can therefore be used to synthesize glucose.

This pathway is also important for ruminant animals, which are dependent on symbiotic microorganisms to break down their food. The microorganisms produce large amounts of propionic acid as a degradation product, which the host can channel into the metabolism in the way described.

Further information ○

In addition to the degradation pathways described above, there are also additional special pathways for particular fatty acids found in food.

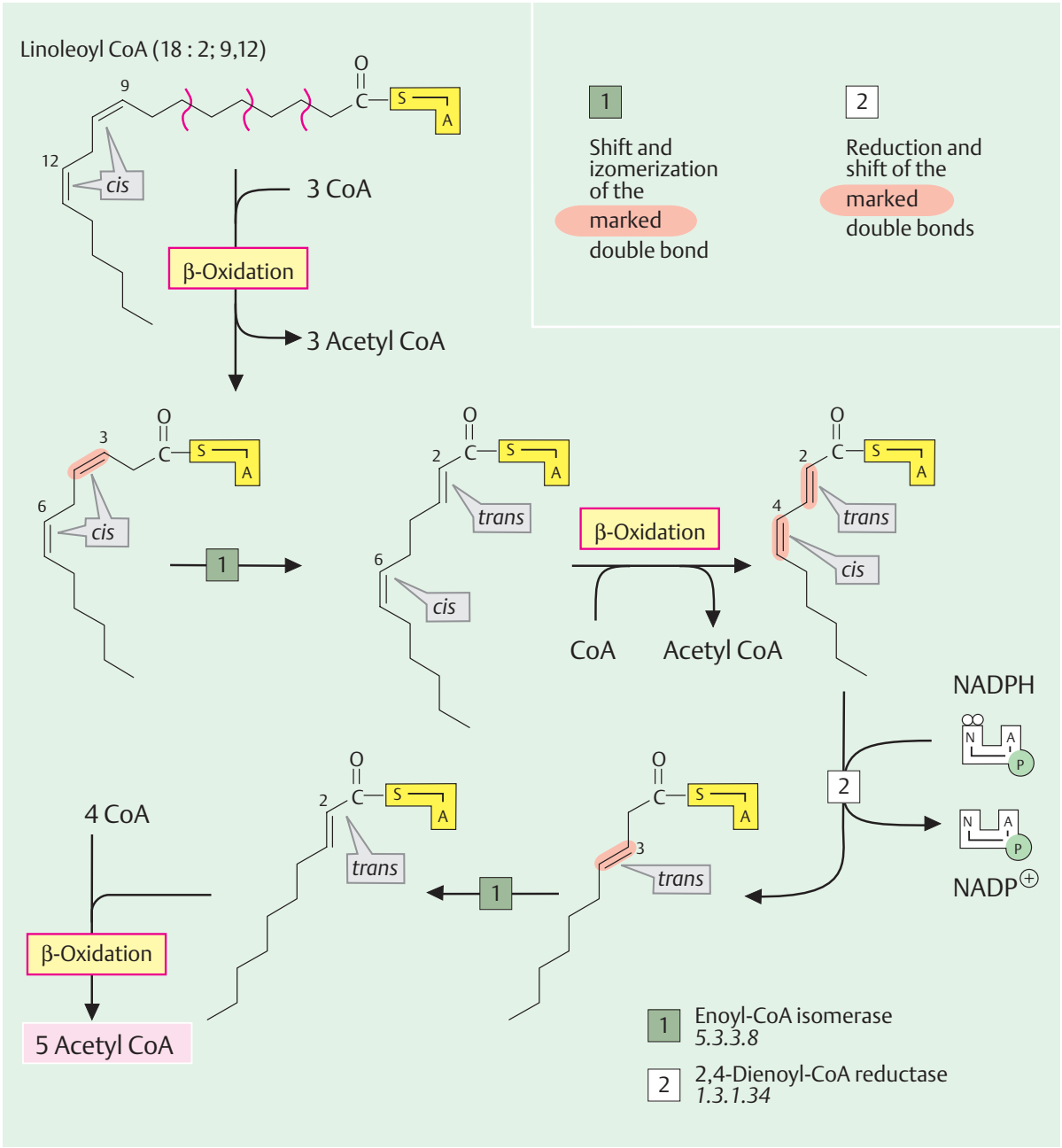
~ Oxidation is used to break down methyl-branched fatty acids. It takes place through step-by-step removal of C_1 residues, begins with a hydroxylation, does not require coenzyme A, and does not produce any ATP.

∂ Oxidation—i. e., oxidation starting at the end of the fatty acid—also starts with a hydroxylation catalyzed by a *monooxygenase* (see p. 316), and leads via subsequent oxidation to fatty acids with two carboxyl groups, which can undergo β-oxidation from both ends until C_8 or C_6 dicarboxylic acids are reached, which can be excreted in the urine in this form.

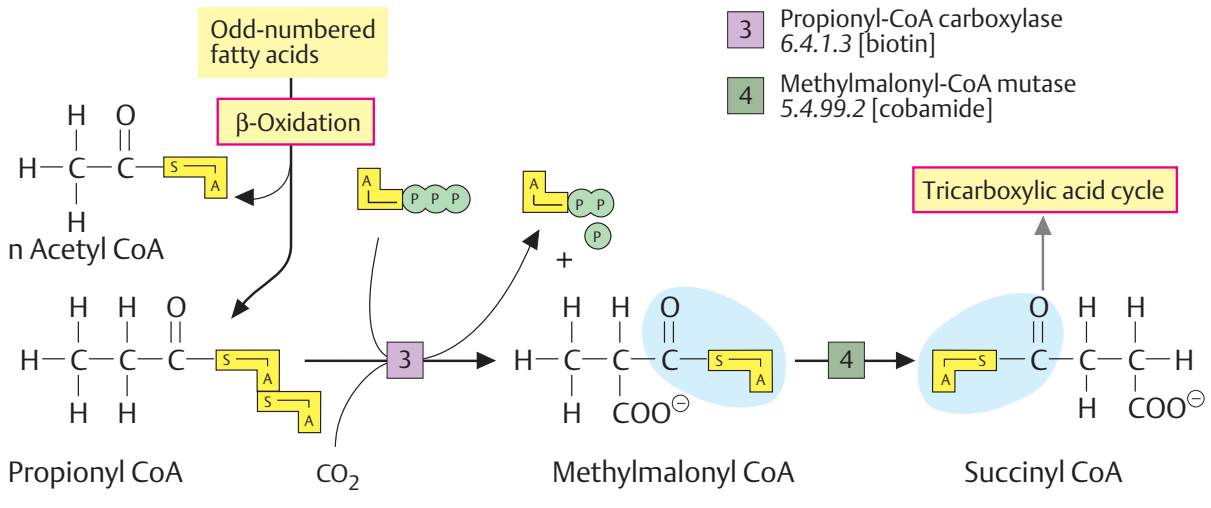
Degradation of unusually long fatty acids. An alternative form of β-oxidation takes place in *hepatic peroxisomes*, which are specialized for the degradation of particularly long fatty acids (n > 20). The degradation products are acetyl-CoA and hydrogen peroxide (H_2O_2), which is detoxified by the *catalase* (see p. 32) common in peroxisomes.

Enzyme defects are also known to exist in the minor pathways of fatty acid degradation. In **Refsum disease**, the methyl-branched phytanic acid (obtained from vegetable foods) cannot be degraded by α-oxidation. In **Zellweger syndrome**, a peroxisomal defect means that long-chain fatty acids cannot be degraded.

A. Degradation of unsaturated fatty acids



B. Degradation of odd-numbered fatty acids



Fatty acid synthesis

In the vertebrates, biosynthesis of fatty acids is catalyzed by *fatty acid synthase,* a multifunctional enzyme. Located in the cytoplasm, the enzyme requires acetyl CoA as a starter molecule. In a cyclic reaction, the acetyl residue is elongated by one C_2 unit at a time for seven cycles. NADPH+H^+ is used as a reducing agent in the process. The end product of the reaction is the saturated C_{16} acid, *palmitic acid.*

A. Fatty acid synthase ◑

Fatty acid synthase in vertebrates consists of two identical peptide chains—i. e., it is a homodimer. Each of the two peptide chains, which are shown here as hemispheres, catalyzes all seven of the partial reactions required to synthesize palmitate. The spatial compression of several successive reactions into a single multifunctional enzyme has advantages in comparison with separate enzymes. Competing reactions are prevented, the individual reactions proceed in a coordinated way as if on a production line, and due to low diffusion losses they are particularly ef cient.

Each subunit of the enzyme binds acetyl residues as thioesters at two different SH groups: at one peripheral *cysteine residue* (CysSH) and one central *4′-phosphopantetheine group* (Pan-SH). Pan-SH, which is very similar to coenzyme A (see p. 12), is covalently bound to a protein segment of the synthase known as the *acyl-carrier protein* (ACP). This part functions like a long arm that passes the substrate from one reaction center to the next. The two subunits of fatty acid synthase cooperate in this process; the enzyme is therefore only capable of functioning as a dimer.

Spatially, the enzyme activities are arranged into three different domains. **Domain 1** catalyzes the entry of the substrates acetyl CoA and malonyl CoA by *[ACP]-S-acetyltransferase* [1] and *[ACP]-Smalonyl transferase* [2] and subsequent condensation of the two partners by *3-oxoacyl-[ACP]-synthase* [3]. **Domain 2** catalyzes the conversion of the 3-oxo group to a CH_2 group by *3-oxoacyl-[ACP]-reductase* [4], *3-hydroxyacyl-[ACP]-dehydratase* [5], and *enoyl-[ACP]-reductase* [6]. Finally, **domain 3** serves to release the finished product by *acyl-[ACP]-hydrolase* [7] after seven steps of chain elongation.

B. Reactions of fatty acid synthase ◑

The key enzyme in fatty acid synthesis is **acetyl CoA carboxylase** (see p. 162), which precedes the synthase and supplies the malonyl-CoA required for elongation. Like all carboxylases, the enzyme contains covalently bound *biotin* as a prosthetic group and is hormone-dependently *inactivated* by phosphorylation or *activated* by dephosphorylation (see p. 120). The precursor *citrate* (see p. 138) is an allosteric activator, while *palmitoyl-CoA* inhibits the end product of the synthesis pathway.

[1] The first cycle (n = 1) starts with the transfer of an acetyl residue from acetyl CoA to the peripheral cysteine residue (Cys-SH). At the same time,

[2] a malonyl residue is transferred from malonyl CoA to 4-phosphopantetheine (Pan-SH).

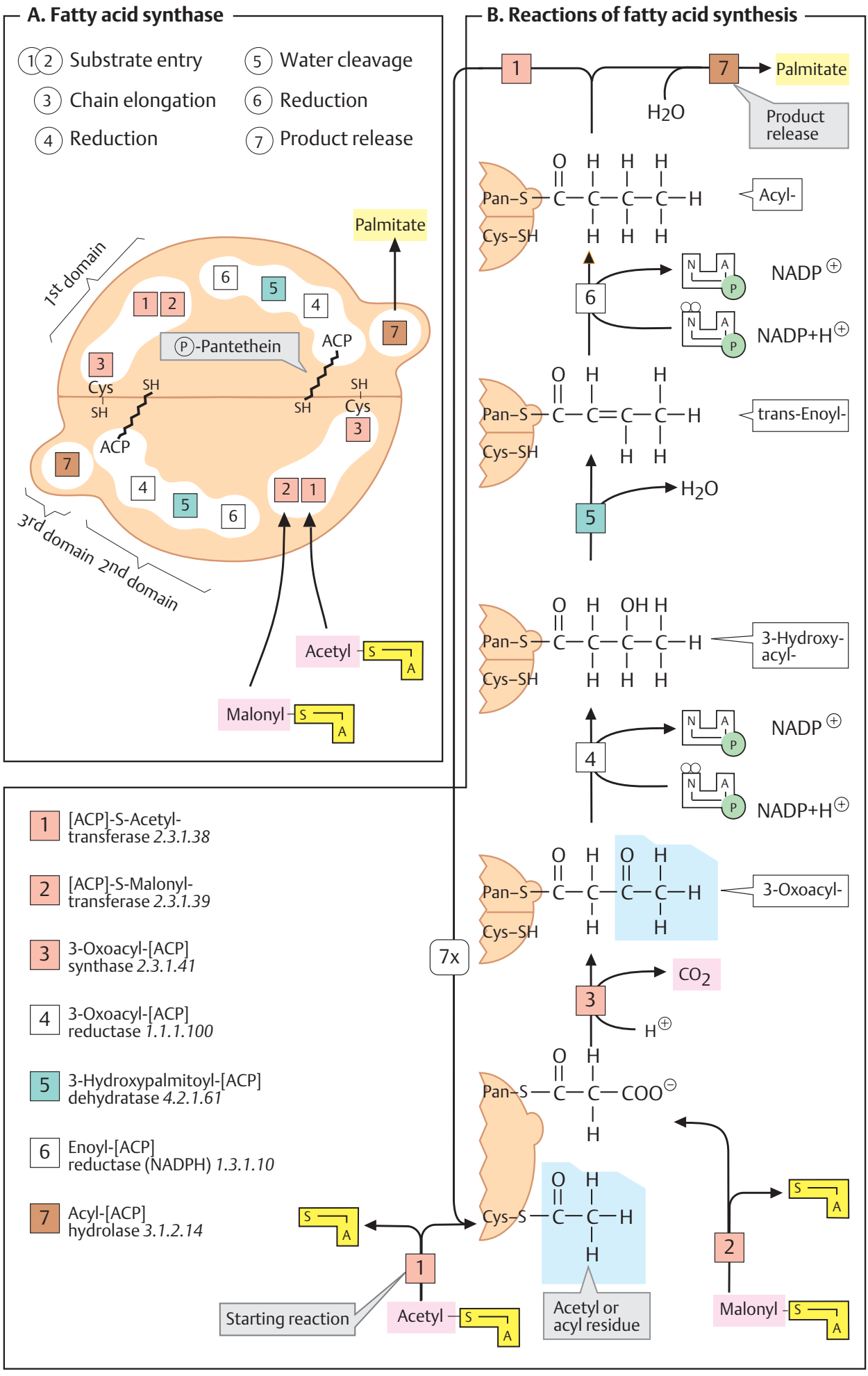
[3] By condensation of the acetyl residue—or (in later cycles) the acyl residue—with the malonyl group, with simultaneous decarboxylation, the chain is elongated.

[4]–[6] The following three reactions (reduction of the 3-oxo group, dehydrogenation of the 3-hydroxyl derivative, and renewed reduction of it) correspond in principle to a reversal of β-oxidation, but they are catalyzed by other enzymes and use NADPH+H^+ instead of NADH+H^+ for reduction. They lead to an acyl residue bound at Pan-SH with 2n + 2 C atoms (n = the number of the cycle). Finally, depending on the length of the product,

[1′] The acyl residue is transferred back to the peripheral cysteine, so that the next cycle can begin again with renewed loading of the ACP with a malonyl residue, or:

[7] After seven cycles, the completed **palmitic acid** is hydrolytically released.

In all, one acetyl-CoA and seven malonyl-CoA are converted with the help of 14 NADPH+H^+ into one palmitic acid, 7 CO_2, 6 H_2O, 8 CoA and 14 $NADP^+$. Acetyl CoA carboxylase also uses up seven ATP.



Biosynthesis of complex lipids

A. Biosynthesis of fats and phospholipids ◑

Complex lipids, such as neutral fats (triacylglycerols), phospholipids, and glycolipids, are synthesized via common reaction pathways. Most of the enzymes involved are associated with the membranes of the smooth endoplasmic reticulum.

The synthesis of fats and phospholipids starts with **glycerol 3-phosphate**. This compound can arise via two pathways:

[1] By reduction from the glycolytic intermediate **glycerone 3-phosphate** (dihydroxyacetone 3-phosphate; enzyme: *glycerol-3-phosphate dehydrogenase (NAD+) 1.1.1.8*), or:

[2] By phosphorylation of **glycerol** deriving from fat degradation (enzyme: *glycerol kinase 2.7.1.30*).

[3] Esterification of glycerol 3-phosphate with a long-chain fatty acid produces a strongly amphipathic **lysophosphatidate** (enzyme: *glycerol-3-phosphate acyltransferase 2.3.1.15*). In this reaction, an acyl residue is transferred from the activated precursor **acyl-CoA** to the hydroxy group at C-1.

[4] A second esterification of this type leads to a **phosphatidate** (enzyme: *1-acylglycerol-3-phosphate acyltransferase 2.3.1.51*). Unsaturated acyl residues, particularly oleic acid, are usually incorporated at C-2 of the glycerol. Phosphatidates (anions of phosphatidic acids) are the key molecules in the biosynthesis of fats, phospholipids, and glycolipids.

[5] To biosynthesize fats (triacylglycerols), the phosphate residue is again removed by hydrolysis (enzyme: *phosphatidate phosphatase 3.1.3.4*). This produces **diacylglycerols (DAG)**.

[6] Transfer of an additional acyl residue to DAG forms **triacylglycerols** (enzyme: *diacylglycerol acyltransferase 2.3.1.20*). This completes the biosynthesis of neutral fats. They are packaged into VLDLs by the liver and released into the blood. Finally, they are stored by adipocytes in the form of insoluble fat droplets.

The biosynthesis of most phospholipids also starts from DAG.

[7] Transfer of a phosphocholine residue to the free OH group gives rise to **phosphatidylcholine** (lecithin; enzyme: *1-alkyl-2-acetylglycerolcholine phosphotransferase 2.7.8.16*). The phosphocholine residue is derived from the precursor CDP-choline (see p. 110). **Phosphatidylethanolamine** is similarly formed from CDP-ethanolamine and DAG. By contrast, **phosphatidylserine** is derived from phosphatidylethanolamine by an exchange of the amino alcohol. Further reactions serve to interconvert the phospholipids—e.g., phosphatidylserine can be converted into phosphatidylethanolamine by decarboxylation, and the latter can then be converted into phosphatidylcholine by methylation with S-adenosyl methionine (not shown; see also p. 409). The biosynthesis of **phosphatidylinositol** starts from phosphatidate rather than DAG.

[8] In the lumen of the intestine, fats from food are mainly broken down into **monoacylglycerols** (see p. 270). The cells of the intestinal mucosa re-synthesize these into neutral fats. This pathway also passes via **DAG** (enzyme: *acylglycerolpalmitoyl transferase 2.3.1.22*).

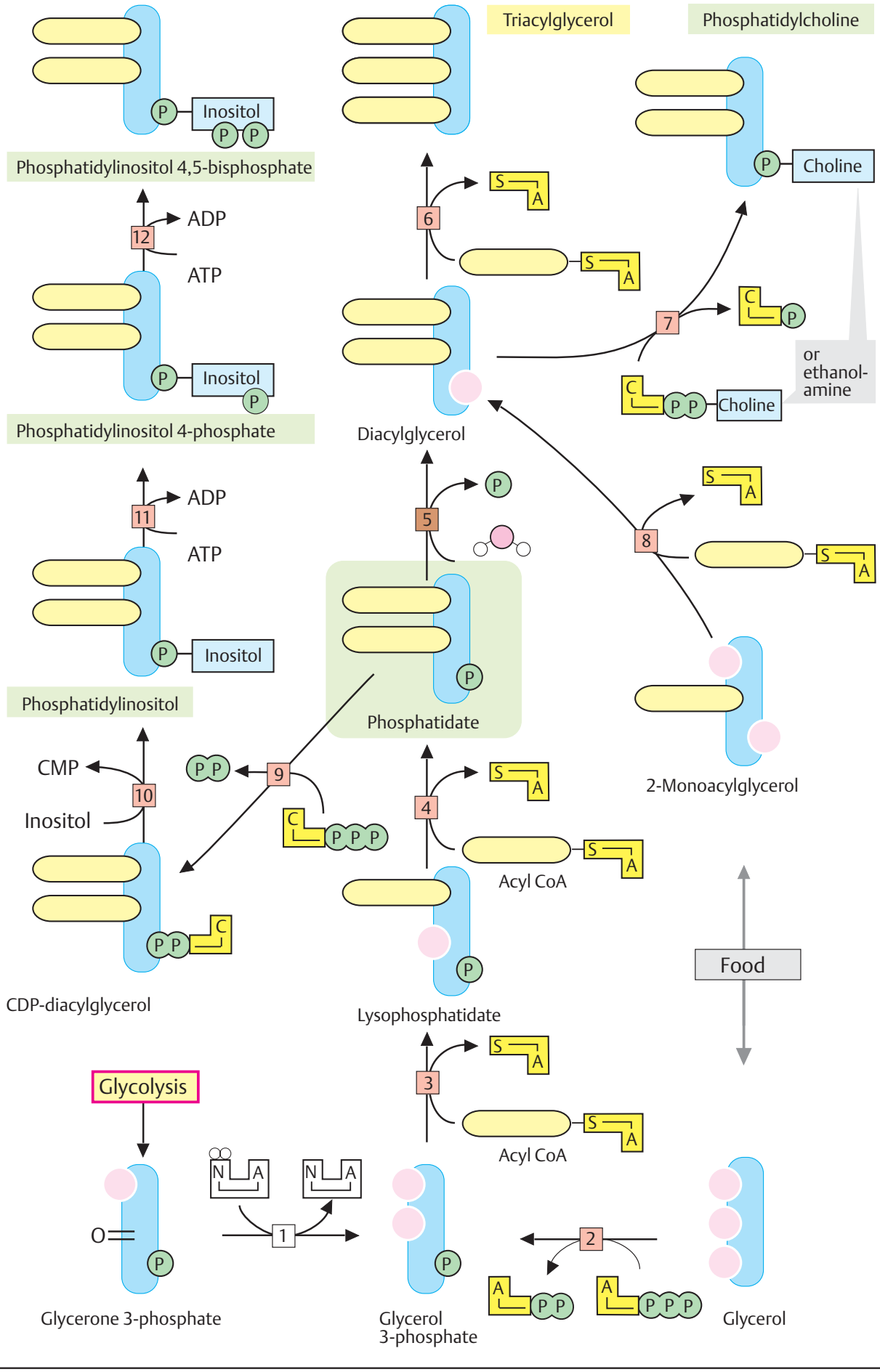
[9] Transfer of a CMP residue gives rise first to **CDP-diacylglycerol** (enzyme: *phosphatidatecytidyl transferase 2.3.1.22*).

[10] Substitution of the CMP residue by inositol then provides **phosphatidylinositol** (**PtdIns**; enzyme: *CDPdiacylglycerolinositol-3-phosphatidyl transferase 2.7.8.11*).

[12] An additional phosphorylation (enzyme: *phosphatidylinositol-4-phosphate kinase 2.7.1.68*) finally provides **phosphaditylinositol-4,5-bisphosphate** (PIP_2, PtdIns(4,5)P_2). PIP_2 is the precursor for the second messengers *2,3-diacylglycerol* (DAG) and *inositol-1,4,5-trisphosphate* ($InsP_3$, IP_3; see p. 367).

The biosynthesis of the **sphingolipids** is shown in schematic form on p. 409.

A. Biosynthesis of fats and phospholipids



Biosynthesis of cholesterol

Cholesterol is a major constituent of the *cell membranes* of animal cells (see p. 216). It would be possible for the body to provide its full daily cholesterol requirement (ca. 1 g) by synthesizing it itself. However, with a mixed diet, only about half of the cholesterol is derived from *endogenous biosynthesis*, which takes place in the intestine and skin, and mainly in the liver (about 50%). The rest is taken up from *food*. Most of the cholesterol is incorporated into the lipid layer of plasma membranes, or converted into **bile acids** (see p. 314). A very small amount of cholesterol is used for biosynthesis of the **steroid hormones** (see p. 376). In addition, up to 1 g cholesterol per day is released into the *bile* and thus excreted.

A. Cholesterol biosynthesis ○

Cholesterol is one of the isoprenoids, synthesis of which starts from **acetyl CoA** (see p. 52). In a long and complex reaction chain, the C_{27} sterol is built up from C_2 components. The biosynthesis of cholesterol can be divided into four sections. In the first (**1**), **mevalonate,** a C_6 compound, arises from three molecules of **acetyl CoA**. In the second part (**2**), mevalonate is converted into **isopentenyl diphosphate,** the "active isoprene." In the third part (**3**), six of these C_5 molecules are linked to produce **squalene,** a C_{30} compound. Finally, squalene undergoes cyclization, with three C atoms being removed, to yield cholesterol (**4**). The illustration only shows the most important intermediates in biosynthesis.

(1) Formation of mevalonate. The conversion of acetyl CoA to acetoacetyl CoA and then to *3-hydroxy-3-methylglutaryl CoA* (3-HMG CoA) corresponds to the biosynthetic pathway for *ketone bodies* (details on p. 312). In this case, however, the synthesis occurs not in the mitochondria as in ketone body synthesis, but in the smooth endoplasmic reticulum. In the next step, the 3-HMG group is cleaved from the CoA and at the same time reduced to mevalonate with the help of $NADPH+H^+$. *3-HMG CoA reductase* is the *key enzyme* in cholesterol biosynthesis. It is regulated by *repression* of transcription (effectors: oxysterols such as cholesterol) and by *interconversion* (effectors: hormones). Insulin and thyroxine stimulate the enzyme and glucagon inhibits it by cAMP-dependent phosphorylation. A large supply of cholesterol from food also inhibits 3-HMG-CoA reductase.

(2) Formation of isopentenyl diphosphate. After phosphorylation, mevalonate is decarboxylated to *isopentenyl diphosphate,* with consumption of ATP. This is the component from which all of the isoprenoids are built (see p. 53).

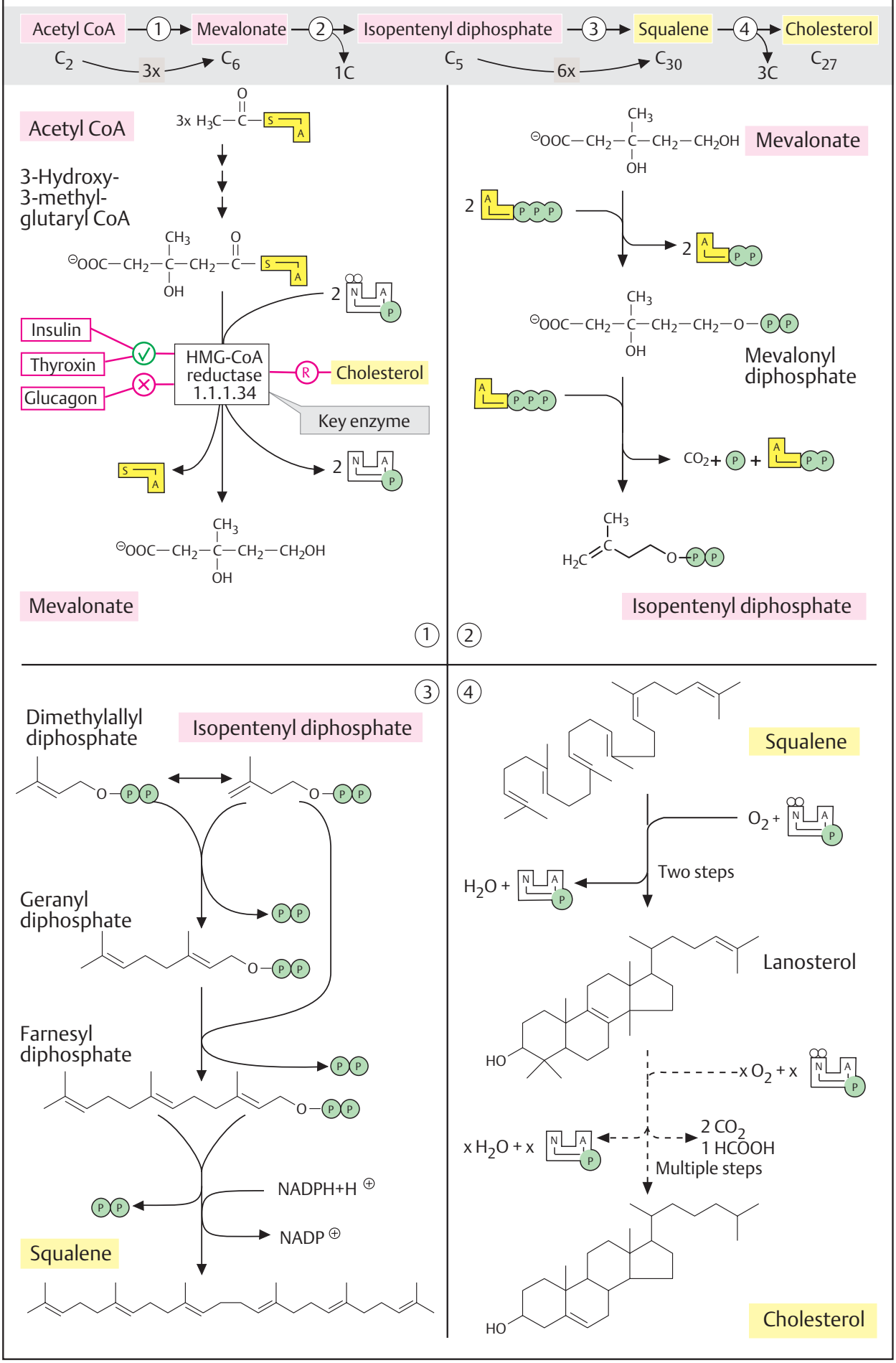
(3) Formation of squalene. Isopentenyl diphosphate undergoes isomerization to form dimethylallyl diphosphate. The two C_5 molecules condense to yield geranyl diphosphate, and the addition of another isopentenyl diphosphate produces farnesyl diphosphate. This can then undergo dimerization, in a *head-to-head reaction,* to yield squalene. Farnesyl diphosphate is also the starting-point for other polyisoprenoids, such as dolichol (see p. 230) and ubiquinone (see p. 52).

(4) Formation of cholesterol. Squalene, a linear isoprenoid, is cyclized, with O_2 being consumed, to form lanosterol, a C_{30} sterol. Three methyl groups are cleaved from this in the subsequent reaction steps, to yield the end product cholesterol. Some of these reactions are catalyzed by *cytochrome P450 systems* (see p. 318).

The endergonic biosynthetic pathway described above is located entirely in the *smooth endoplasmic reticulum.* The energy needed comes from the CoA derivatives used and from ATP. The reducing agent in the formation of mevalonate and squalene, as well as in the final steps of cholesterol biosynthesis, is $NADPH+H^+$.

The division of the intermediates of the reaction pathway into three groups is characteristic: CoA compounds, diphosphates, and highly lipophilic, poorly soluble compounds (squalene to cholesterol), which are bound to *sterol carriers* in the cell.

A. Cholesterol biosynthesis



Protein metabolism: overview

Quantitatively, proteins are the most important group of endogenous macromolecules. A person weighing 70 kg contains about 10 kg protein, with most of it located in muscle. By comparison, the proportion made up by other nitrogencontaining compounds is minor. The organism's nitrogen balance is therefore primarily determined by protein metabolism. Several hormones—mainly *testosterone* and *cortisol*—regulate the nitrogen balance (see p. 374).

A. Protein metabolism: overview ●

In adults, the **nitrogen balance** is generally in *equilibrium*—i. e., the quantities of protein nitrogen taken in and excreted per day are approximately equal. If only some of the nitrogen taken in is excreted again, then the balance is *positive*. This is the case during growth, for example. *Negative* balances are rare and usually occur due to disease.

Proteins taken up in food are initially broken down in the gastrointestinal tract into amino acids, which are resorbed and distributed in the organism via the blood (see p. 266). The human body is not capable of synthesizing 8–10 of the 20 proteinogenic amino acids it requires (see p. 60). These amino acids are **essential**, and have to be supplied from food (see p. 184).

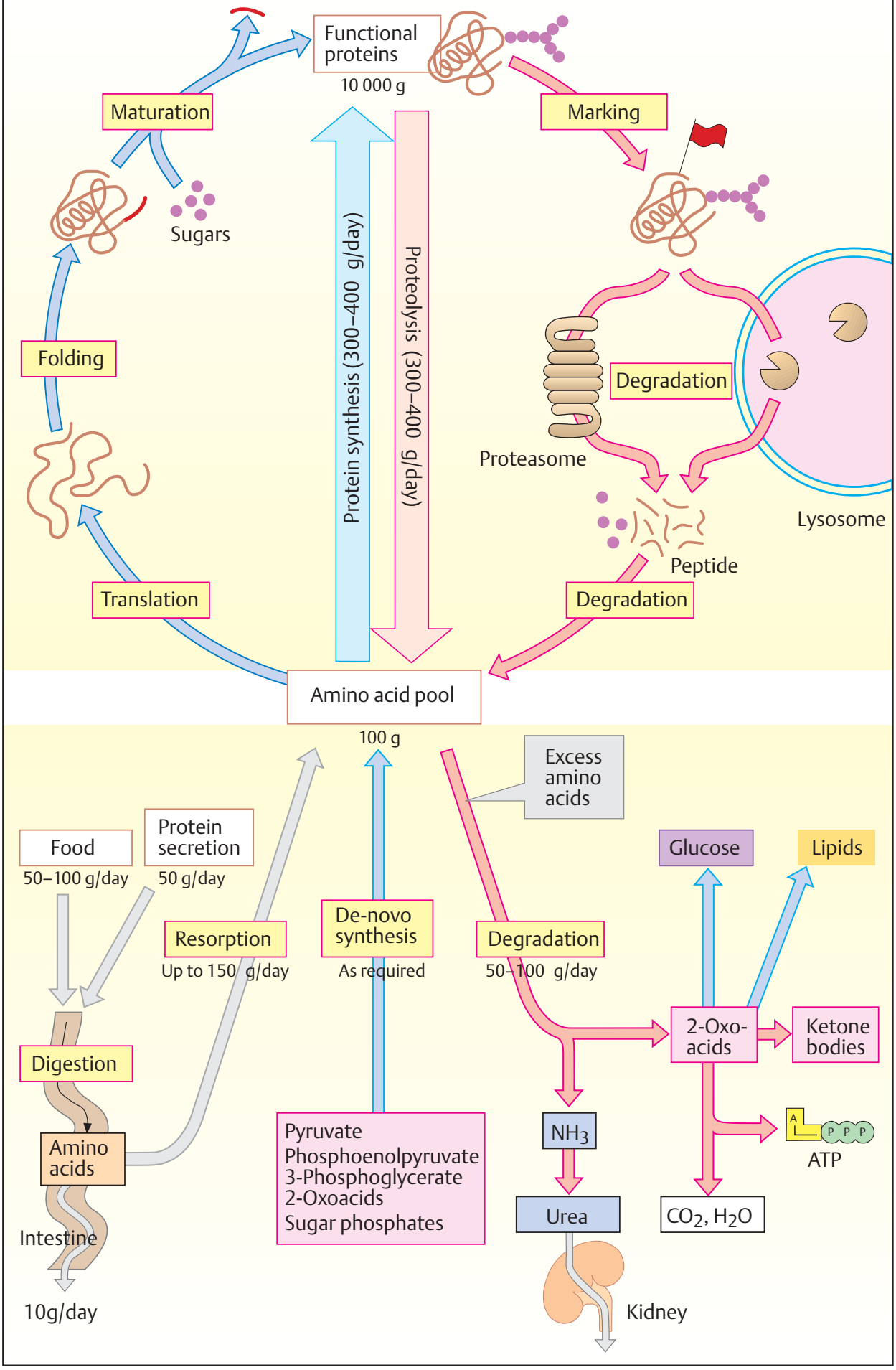
Proteins are constantly being lost via the intestine and, to a lesser extent, via the kidneys. To balance these inevitable losses, at least 30 g of protein have to be taken up with food every day. Although this minimum value is barely reached in some countries, in the industrial nations the protein content of food is usually much higher than necessary. As it is not possible to store amino acids, up to 100 g of excess amino acids per day are used for biosynthesis or degraded in the liver in this situation. The nitrogen from this excess is converted into urea (see p. 182) and excreted in the urine in this form. The carbon skeletons are used to synthesize carbohydrates or lipids (see p. 180), or are used to form ATP.

It is thought that adults break down 300–400 g of protein per day into amino acids (**proteolysis**). On the other hand, approximately the same amount of amino acids is reincorporated into proteins (**protein biosynthesis**). The body's high level of protein turnover is due to the fact that many proteins are relatively *short-lived*. On average, their half-lives amount to 2–8 days. The *key enzymes* of the intermediary metabolism have even shorter half-lives. They are sometimes broken down only a few hours after being synthesized, and are replaced by new molecules. This constant process of synthesis and degradation makes it possible for the cells to quickly adjust the quantities, and therefore the activity, of important enzymes in order to meet current requirements. By contrast, structural proteins such as the histones, hemoglobin, and the components of the cytoskeleton are particularly long-lived.

Almost all cells are capable of carrying out **biosynthesis** of proteins (top left). The formation of peptide chains by **translation** at the ribosome is described in greater detail on pp. 250–253. However, the functional forms of most proteins arise only after a series of additional steps. To begin with, supported by auxiliary proteins, the biologically active conformation of the peptide chain has to be formed (**folding**; see pp. 74, 232). During subsequent "post-translational" **maturation**, many proteins remove part of the peptide chain again and attach additional groups—e. g., oligosaccharides or lipids. These processes take place in the endoplasmic reticulum and in the Golgi apparatus (see p. 232). Finally, the proteins have to be transported to their site of action (**sorting**; see p. 228).

Some *intracellular* protein degradation (**proteolysis**) takes place in the lysosomes (see p. 234). In addition, there are protein complexes in the cytoplasm, known as *proteasomes,* in which incorrectly folded or old proteins are degraded. These molecules are recognized by a special **marking** (see p. 176). The proteasome also plays an important part in the presentation of antigens by immune cells (see p. 296).

A. Protein metabolism: overview



Proteolysis

A. Proteolytic enzymes ◑

Combinations of several enzymes with different specificities are required for complete degradation of proteins into free amino acids. **Proteinases** and **peptidases** are found not only in the gastrointestinal tract (see p. 268), but also inside the cell (see below).

The proteolytic enzymes are classified into **endopeptidases** and **exopeptidases**, according to their site of attack in the substrate molecule. The *endopeptidases* or *proteinases* cleave peptide bonds *inside* peptide chains. They "recognize" and bind to short sections of the substrate's sequence, and then hydrolyze bonds between particular amino acid residues in a relatively specific way (see p. 94). The **proteinases** are classified according to their reaction mechanism. In *serine proteinases,* for example (see **C**), a serine residue in the enzyme is important for catalysis, while in *cysteine proteinases,* it is a cysteine residue, and so on.

The exopeptidases attack peptides from their termini. Peptidases that act at the N terminus are known as **aminopeptidases**, while those that recognize the C terminus are called **carboxypeptidases**. The **dipeptidases** only hydrolyze dipeptides.

B. Proteasome ○

The functional proteins in the cell have to be protected in order to prevent premature degradation. Some of the intracellularly active proteolytic enzymes are therefore enclosed in lysosomes (see p. 234). The proteinases that act there are also known as **cathepsins**. Another carefully regulated system for protein degradation is located in the cytoplasm. This consists of large protein complexes (mass 2 10^6 Da), the **proteasomes**. Proteasomes contain a barrel-shaped core consisting of 28 subunits that has a sedimentation coef cient (see p. 200) of 20 S. Proteolytic activity (shown here by the scissors) is localized in the interior of the 20-S core and is therefore protected. The openings in the barrel are sealed by 19-S particles with a complex structure that control access to the core.

Proteins destined for degradation in the proteasome (e. g., incorrectly folded or old molecules) are marked by covalent linkage with chains of the small protein **ubiquitin**. The ubiquitin is previously activated by the introduction of reactive thioester groups. Molecules marked with ubiquitin ("ubiquitinated") are recognized by the 19S particle, unfolded using ATP, and then shifted into the interior of the nucleus, where degradation takes place. Ubiquitin is not degraded, but is reused after renewed activation.

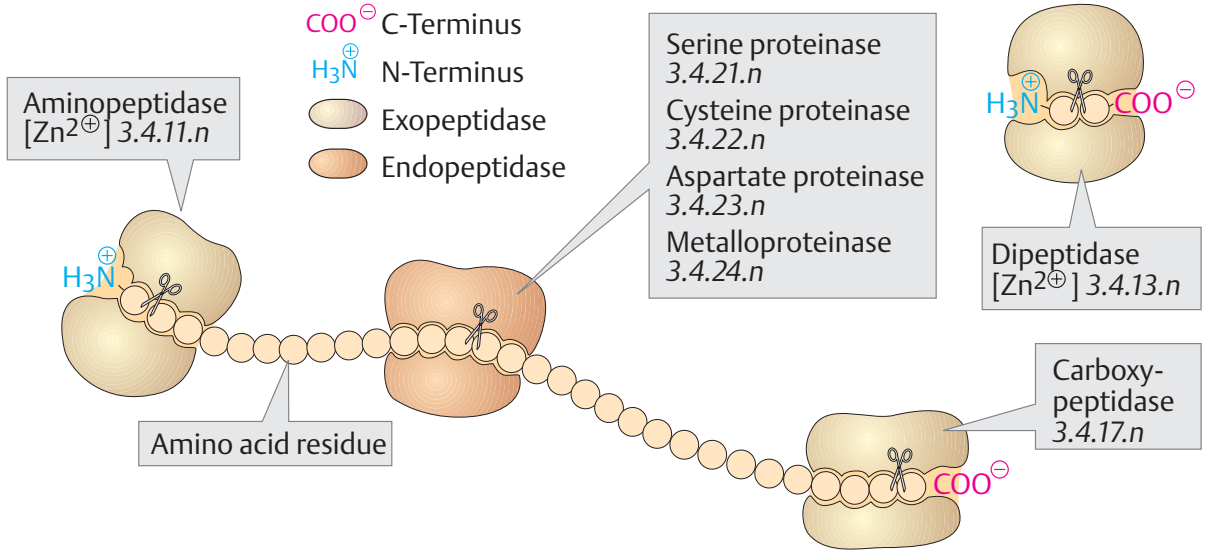
C. Serine proteases ○

A large group of proteinases contain serine in their active center. The serine proteases include, for example, the digestive enzymes *trypsin, chymotrypsin,* and *elastase* (see pp. 94 and 268), many *coagulation factors* (see p. 290), and the fibrinolytic enzyme *plasmin* and its *activators* (see p. 292).

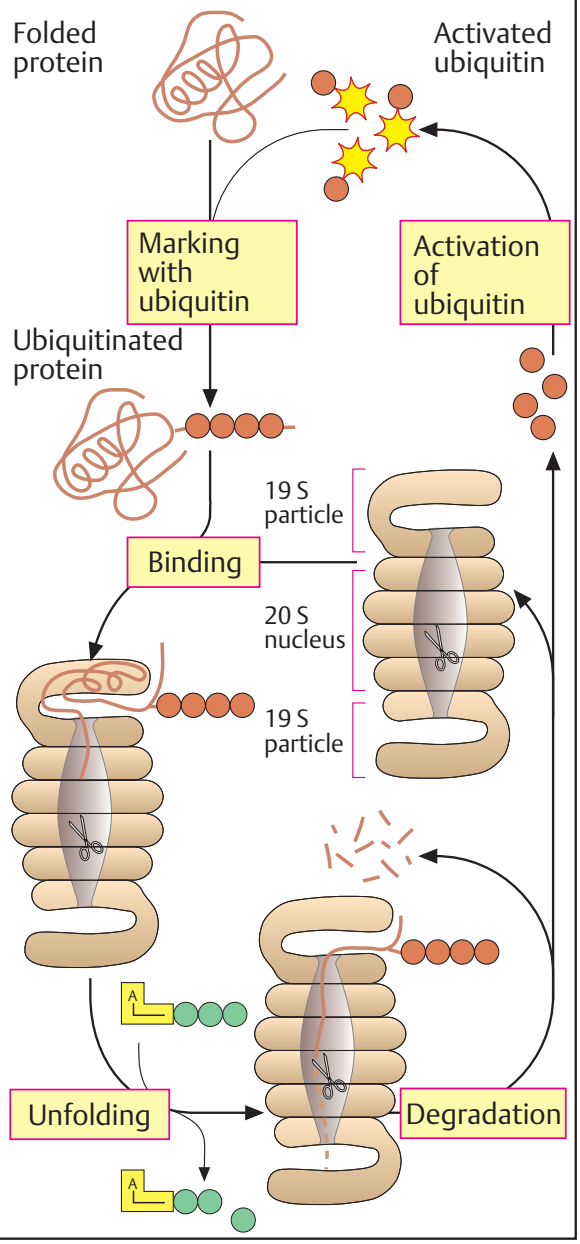
As described on p. 270, pancreatic proteinases are secreted as **proenzymes** (zymogens). Activation of these is also based on proteolytic cleavages. This is illustrated here in detail using the example of **trypsinogen**, the precursor of trypsin (**1**). Activation of trypsinogen starts with cleavage of an N-terminal hexapeptide by *enteropeptidase* (enterokinase), a specific serine proteinase that is located in the membrane of the intestinal epithelium. The cleavage product (β-trypsin) is already catalytically active, and it cleaves additional trypsinogen molecules at the sites marked in red in the illustration (autocatalytic cleavage). The precursors of chymotrypsin, elastase, and carboxypeptidase A, among others, are also activated by trypsin.

The active center of trypsin is shown in Fig. **2**. A serine residue in the enzyme (Ser-195), supported by a histidine residue and an aspartate residue (His-57, Asp-102), nucleophilically attacks the bond that is to be cleaved (red arrow). The cleavage site in the substrate peptide is located on the C-terminal side of a lysine residue, the side chain of which is fixed in a special "binding pocket" of the enzyme (left) during catalysis (see p. 94).

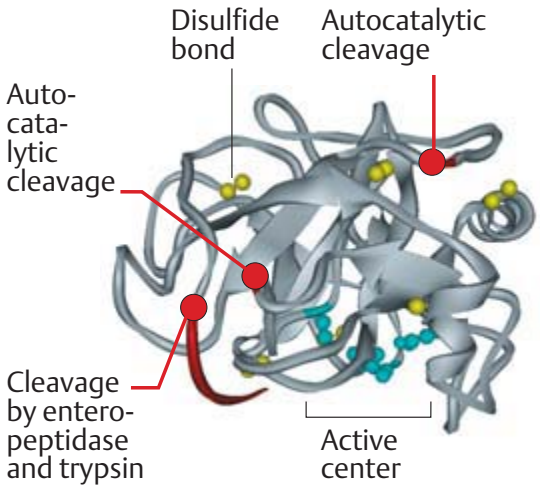
A. Proteolytic enzymes



B. Proteasome



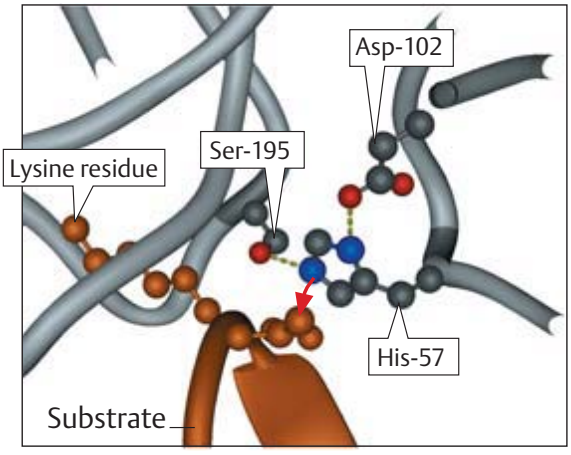
C. Serine proteases



1 Enteropeptidase *3.4.21.9*

2 Trypsin *3.4.21.4*

1. Trypsinogen activation



2. Trypsin: active center

Transamination and deamination

Amino nitrogen accumulates during protein degradation. In contrast to carbon, amino nitrogen is not suitable for oxidative energy production. If they are not being reused for biosynthesis, the amino groups of amino acids are therefore incorporated into urea (see p. 182) and excreted in this form.

A. Transamination and deamination ◑

Among the NH_2 transfer reactions, **transaminations** (**1**) are particularly important. They are catalyzed by *transaminases*, and occur in both catabolic and anabolic amino acid metabolism. During transamination, the amino group of an amino acid (amino acid 1) is transferred to a 2-oxoacid (oxoacid 2). From the amino acid, this produces a 2-oxoacid (a), while from the original oxoacid, an amino acid is formed (b). The NH_2 group is temporarily taken over by enzyme-bound **pyridoxal phosphate** (PLP; see p. 106), which thus becomes pyridoxamine phosphate.

If the NH_2 is released as ammonia, the process is referred to as **deamination**. There are different mechanisms for this (see p. 180). A particularly important one is **oxidative deamination** (**2**). In this reaction, the α-amino group is initially *oxidized* into an imino group (2a), and the reducing equivalents are transferred to NAD^+ or $NADP^+$. In the second step, the imino group is then cleaved by *hydrolysis*. As in transamination, this produces a 2-oxoacid (**C**). Oxidative deamination mainly takes place in the liver, where glutamate is broken down in this way into 2-oxoglutarate and ammonia, catalyzed by *glutamate dehydrogenase.* The reverse reaction initiates biosynthesis of the amino acids in the glutamate family (see p. 184).

B. Mechanism of transamination ○

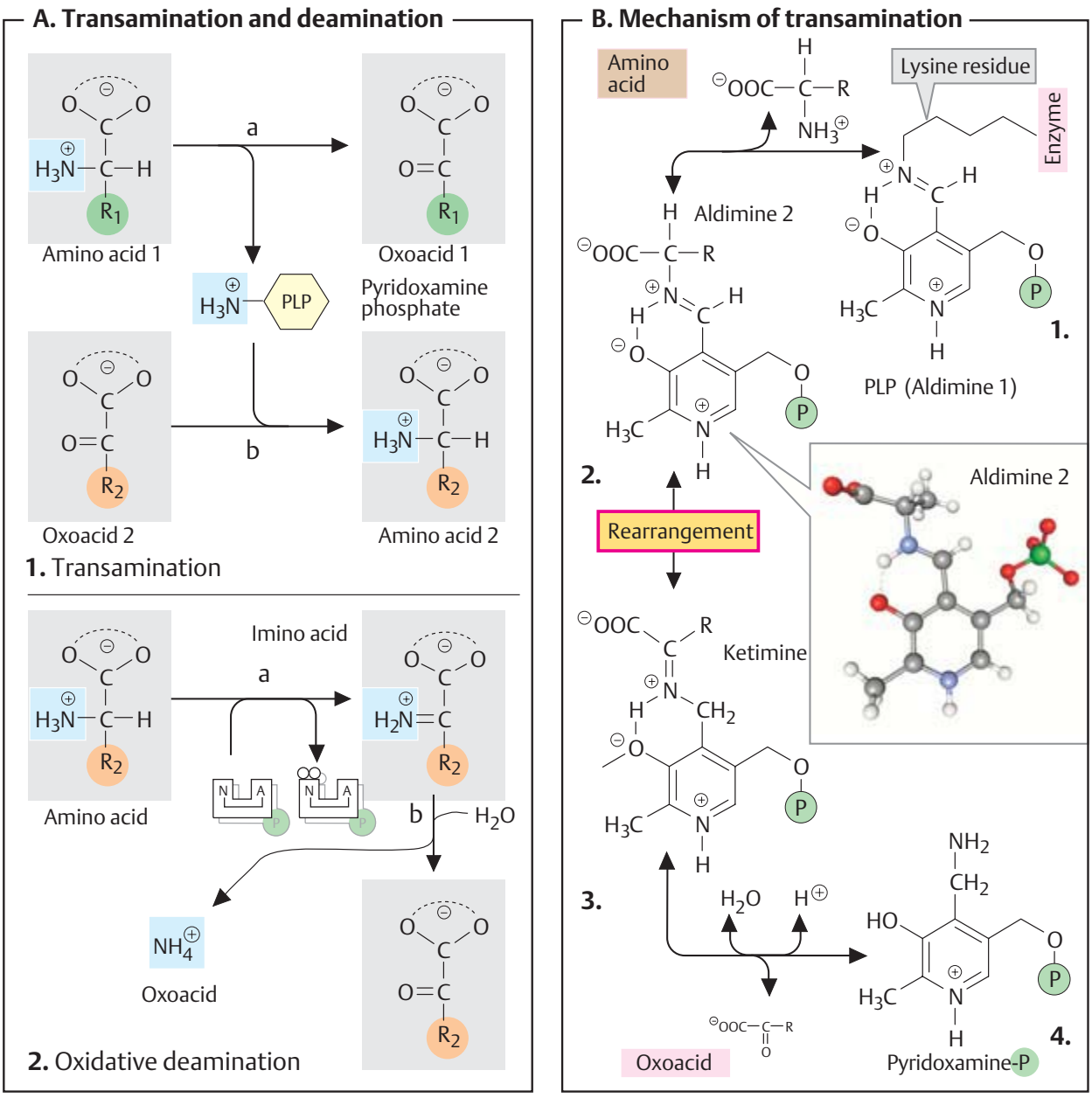
In the absence of substrates, the aldehyde group of pyridoxal phosphate is covalently bound to a lysine residue of the transaminase (**1**). This type of compound is known as an **aldimine** or "Schiff's base." During the reaction, amino acid 1 (**A**, **1a**) displaces the lysine residue, and a new aldimine is formed (**2**). The double bond is then shifted by isomerization. The ketimine (**3**) is hydrolyzed to yield the 2-oxoacid and **pyridoxamine phosphate** (**4**).

In the second part of the reaction (see **A**, 1b), these steps take place *in the opposite direction:* pyridoxamine phosphate and the second 2-oxoacid form a ketimine, which is isomerized into aldimine. Finally, the second amino acid is cleaved and the coenzyme is regenerated.

C. NH_3 metabolism in the liver ◑

In addition to urea synthesis itself (see p. 182), the precursors NH_3 and aspartate are also mainly formed in the liver. Amino nitrogen arising in tissue is transported to the liver by the blood, mainly in the form of **glutamine** (Gln) and **alanine** (Ala; see p. 338). In the liver, Gln is hydrolytically deaminated by *glutaminase* [3] into **glutamate** (Glu) and NH_3. The amino group of the alanine is transferred by *alanine transaminase* [1] to **2-oxoglutarate** (2-OG; formerly known as α-ketoglutarate). This transamination (**A**) produces another glutamate. NH_3 is finally released from glutamate by oxidative deamination (**A**). This reaction is catalyzed by *glutamate dehydrogenase* [4], a typical liver enzyme. **Aspartate** (Asp), the second amino group donor in the urea cycle, also arises from glutamate. The *aspartate transaminase* [2] responsible for this reaction is found with a high level of activity in the liver, as is *alanine transaminase* [1].

Transaminases are also found in other tissues, from which they leak from the cells into the blood when injury occurs. Measurement of serum enzyme activity (**serum enzyme diagnosis**; see also p. 98) is an important method of recognizing and monitoring the course of such injuries. Transaminase activity in the blood is for instance important for diagnosing liver disease (e.g., hepatitis) and myocardial disease (cardiac infarction).



Amino acid degradation

A large number of metabolic pathways are available for amino acid degradation, and an overview of these is presented here. Further details are given on pp. 414 and 415.

A. Amino acid degradation : overview ◑

During the degradation of most amino acids, the α-amino group is initially removed by **transamination** or **deamination**. Various mechanisms are available for this, and these are discussed in greater detail in **B**. The carbon skeletons that are left over after deamination undergo further degradation in various ways.

During degradation, the 20 proteinogenic amino acids produce only seven different **degradation products** (highlighted in pink and violet). Five of these metabolites (2-oxoglutarate, succinyl CoA, fumarate, oxaloacetate, and pyruvate) are precursors for gluconeogenesis and can therefore be converted into glucose by the liver and kidneys (see p. 154). Amino acids whose degradation supplies one of these five metabolites are therefore referred to as **glucogenic amino acids**. The first four degradation products listed are already intermediates in the tricarboxylic acid cycle, while pyruvate can be converted into oxaloacetate by *pyruvate carboxylase* and thus made available for gluconeogenesis (green arrow).

With two exceptions (lysine and leucine; see below), all of the proteinogenic amino acids are also glucogenic. Quantitatively, they represent the most important precursors for gluconeogenesis. At the same time, they also have an **anaplerotic** effect—i. e., they replenish the tricarboxylic acid cycle in order to feed the anabolic reactions that originate in it (see p. 138).

Two additional degradation products (acetoacetate and acetyl CoA) cannot be channeled into gluconeogenesis in animal metabolism, as there is no means of converting them into precursors of gluconeogenesis. However, they can be used to synthesize ketone bodies, fatty acids, and isoprenoids. Amino acids that supply acetyl CoA or acetoacetate are therefore known as **ketogenic amino acids.** Only leucine and lysine are *purely* ketogenic. Several amino acids yield degradation products that are both *glucogenic and ketogenic*. This group includes phenylalanine, tyrosine, tryptophan, and isoleucine.

Degradation of acetoacetate to acetyl CoA takes place in two steps (not shown). First, acetoacetate and succinyl CoA are converted into acetoacetyl CoA and succinate (enzyme: *3-oxoacid-CoA transferase 2.8.3.5*). Acetoacetyl CoA is then broken down by β-oxidation into two molecules of acetyl CoA (see p. 164), while succinate can be further metabolized via the tricarboxylic acid cycle.

B. Deamination ○

There are various ways of releasing ammonia (NH_3) from amino acids, and these are illustrated here using the example of the amino acids glutamine, glutamate, alanine, and serine.

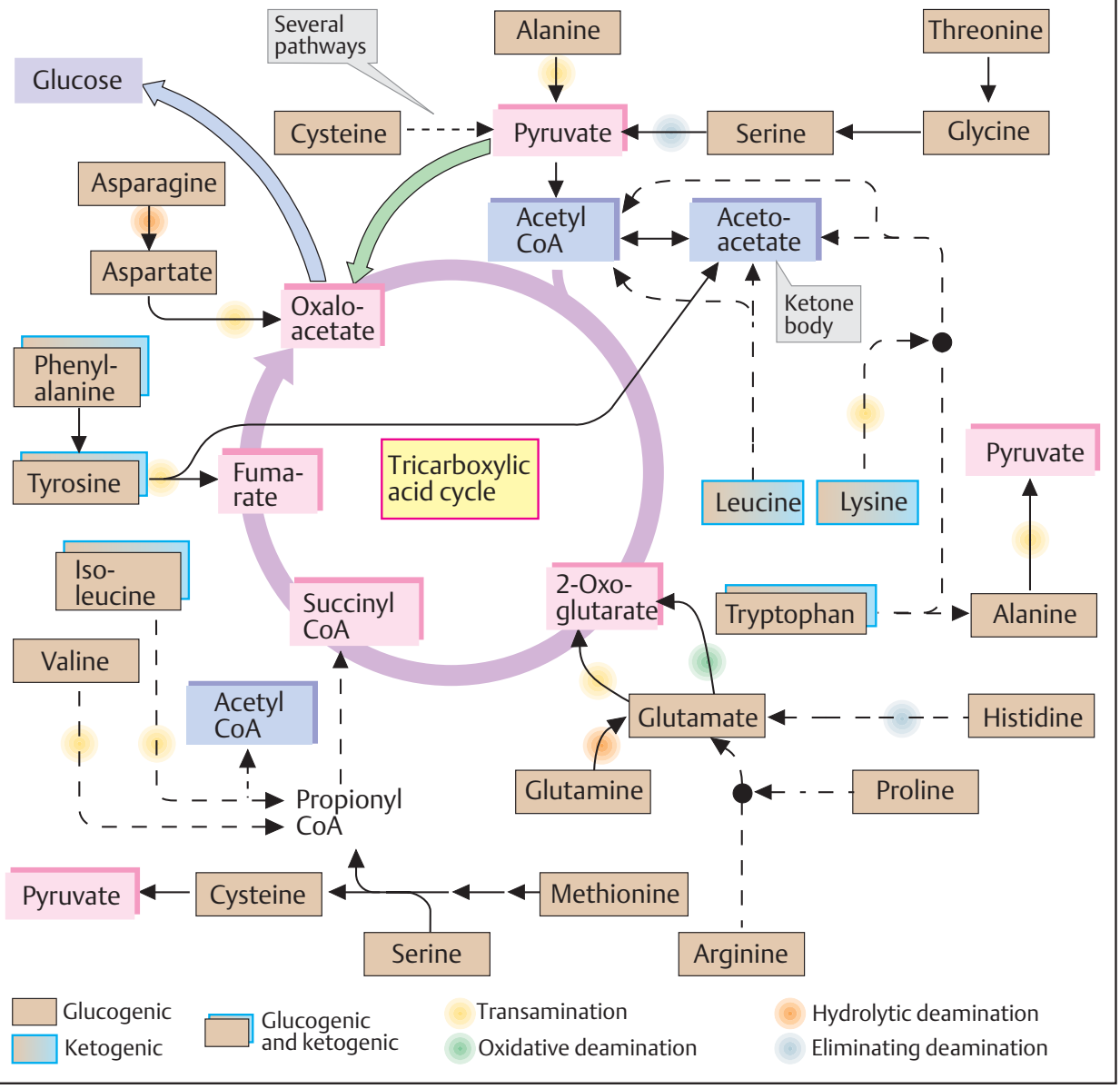
[1] In the branched-chain amino acids (Val, Leu, Ile) and also tyrosine and ornithine, degradation starts with a **transamination**. For alanine and aspartate, this is actually the only degradation step. The mechanism of transamination is discussed in detail on p. 178.

[2] **Oxidative deamination**, with the formation of $NADH+H^+$, only applies to glutamate in animal metabolism. The reaction mainly takes place in the liver and releases NH_3 for urea formation (see p. 178).

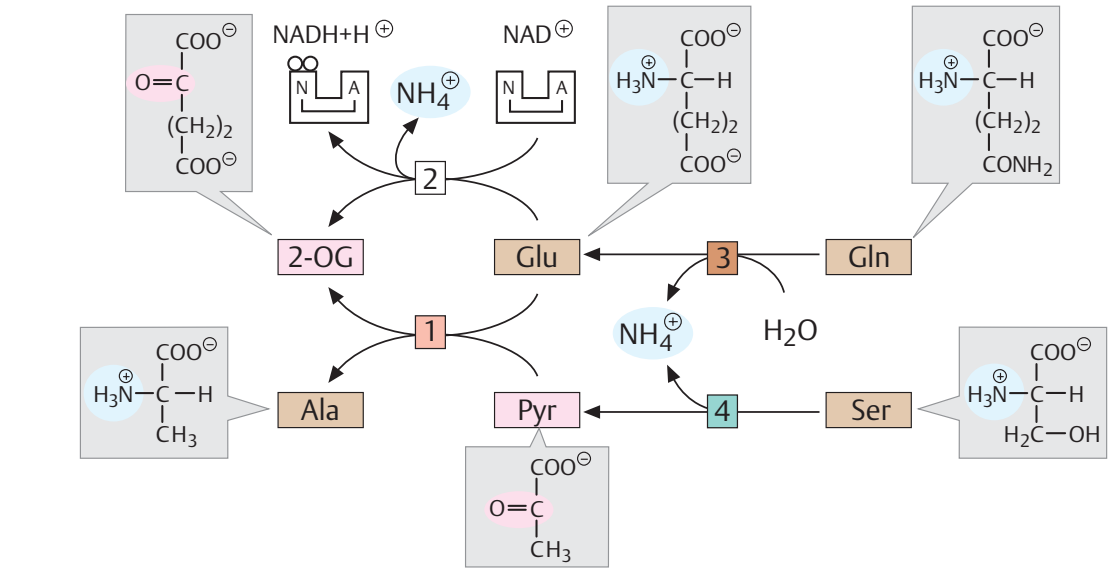
[3] Two amino acids—asparagine and glutamine—contain acid–amide groups in the side chains, from which NH_3 can be released by hydrolysis (**hydrolytic deamination**). In the blood, glutamine is the most important transport molecule for amino nitrogen. Hydrolytic deamination of glutamine in the liver also supplies the urea cycle with NH_3.

[4] **Eliminating deamination** takes place in the degradation of histidine and serine. H_2O is first eliminated here, yielding an unsaturated intermediate. In the case of serine, this intermediate is first rearranged into an imine (not shown), which is hydrolyzed in the second step into NH_3 and pyruvate, with H_2O being taken up. H_2O does not therefore appear in the reaction equation.

A. Amino acid degradation: overview



B. Deamination



1 Alanine transaminase [PLP] *2.6.1.2*
2 Glutamate dehydrogenase *1.4.1.2*
3 Glutaminase *3.5.1.2*
4 Serine dehydratase [PLP] *4.2.1.13*

Urea cycle

Amino acids are mainly broken down in the liver. Ammonia is released either directly or indirectly in the process (see p. 178). The degradation of nucleobases also provides significant amounts of ammonia (see p. 186).

Ammonia (NH_3) is a relatively strong **base**, and at physiological pH values it is mainly present in the form of the **ammonium ion** $\mathbf{NH_4^+}$ (see p. 30). NH_3 and NH_4^+ are toxic, and at higher concentrations cause brain damage in particular. Ammonia therefore has to be effectively inactivated and excreted. This can be carried out in various ways. Aquatic animals can excrete NH_4^+ directly. For example, fish excrete NH_4^+ via the gills (*ammonotelic animals*). Terrestrial vertebrates, including humans, hardly excrete any NH_3, and instead, most ammonia is converted into urea before excretion (*ureotelic animals*). Birds and reptiles, by contrast, form *uric acid,* which is mainly excreted as a solid in order to save water (*uricotelic animals*).

The reasons for the neurotoxic effects of ammonia have not yet been explained. It may disturb the metabolism of glutamate and its precursor glutamine in the brain (see p. 356).

A. Urea cycle ◑

Urea ($H_2N–CO–NH_2$) is the diamide of carbonic acid. In contrast to ammonia, it is **neutral** and therefore relatively **non-toxic**. The reason for the lack of basicity is the molecule's mesomeric characteristics. The free electron pairs of the two nitrogen atoms are *delocalized* over the whole structure, and are therefore no longer able to bind protons. As a small, uncharged molecule, urea is able to cross biological membranes easily. In addition, it is easily transported in the blood and excreted in the urine.

Urea is produced **only in the liver**, in a cyclic sequence of reactions (the **urea cycle**) that starts in the mitochondria and continues in the cytoplasm. The two nitrogen atoms are derived from $\mathbf{NH_4^+}$ (the second has previously been incorporated into aspartate; see below). The keto group comes from **hydrogen carbonate** (HCO_3^-), or CO_2 that is in equilibrium with HCO_3^-.

[1] In the first step, **carbamoyl phosphate** is formed in the mitochondria from hydrogen carbonate (HCO_3^-) and NH_4^+, with two ATP molecules being consumed. In this compound, the carbamoyl residue ($–O–CO–NH_2$) is at a high chemical potential. In hepatic mitochondria, enzyme [1] makes up about 20% of the matrix proteins.

[2] In the next step, the carbamoyl residue is transferred to the non-proteinogenic amino acid **ornithine**, converting it into **citrulline**, which is also non-proteinogenic. This is passed into the cytoplasm via a transporter.

[3] The second NH_2 group of the later urea molecule is provided by **aspartate**, which condenses with citrulline into **argininosuccinate**. ATP is cleaved into AMP and diphosphate (PP_i) for this endergonic reaction. To shift the equilibrium of the reaction to the side of the product, diphosphate is removed from the equilibrium by hydrolysis.

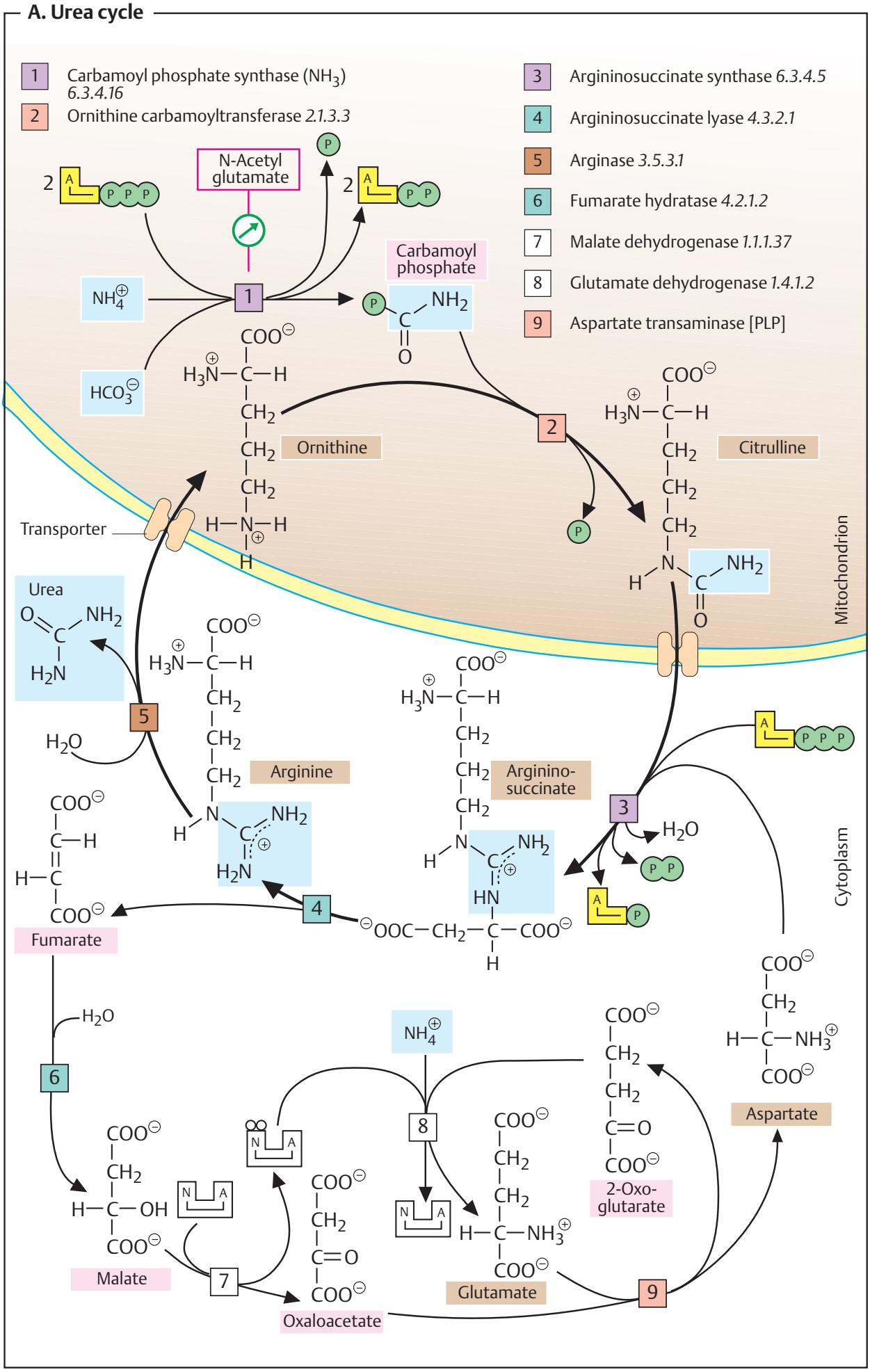
[4] Cleavage of fumarate from argininosuccinate leads to the proteinogenic amino acid **arginine**, which is synthesized in this way in animal metabolism.

[5] In the final step, isourea is released from the guanidinium group of the arginine by hydrolysis (not shown), and is immediately rearranged into **urea**. In addition, ornithine is regenerated and returns via the ornithine transporter into the mitochondria, where it becomes available for the cycle once again.

The **fumarate** produced in step [4] is converted via malate to oxaloacetate [6, 7], from which **aspartate** is formed again by transamination [9]. The glutamate required for reaction [9] is derived from the glutamate dehydrogenase reaction [8], which fixes the second NH_4^+ in an organic bond. Reactions [6] and [7] also occur in the tricarboxylic acid cycle. However, in urea formation they take place in the cytoplasm, where the appropriate isoenzymes are available.

The rate of urea formation is mainly controlled by reaction [1]. *N*-**acetyl glutamate**, as an allosteric effector, activates *carbamoylphosphate synthase.* In turn, the concentration of acetyl glutamate depends on arginine and ATP levels, as well as other factors.

A. Urea cycle



Amino acid biosynthesis

A. Symbiotic nitrogen fixation ○

Practically unlimited quantities of elementary nitrogen (N_2) are present in the atmosphere. However, before it can enter the natural nitrogen cycle, it has to be reduced to NH_3 and incorporated into amino acids ("fixed"). Only a few species of bacteria and bluegreen algae are capable of fixing atmospheric nitrogen. These exist freely in the soil, or in **symbiosis** with plants. The symbiosis between bacteria of the genus *Rhizobium* and legumes (*Fabales*)—such as clover, beans, and peas—is of particular economic importance. These plants are high in protein and are therefore nutritionally valuable.

In symbiosis with *Fabales*, bacteria live as *bacteroids* in **root nodules** inside the plant cells. The plant supplies the bacteroids with nutrients, but it also benefits from the fixed nitrogen that the symbionts make available.

The N_2-fixing enzyme used by the bacteria is *nitrogenase*. It consists of two components: an *Fe protein* that contains an $[Fe_4S_4]$ cluster as a redox system (see p. 106), accepts electrons from *ferredoxin*, and donates them to the second component, the *Fe–Mo protein*. This molybdenum-containing protein transfers the electrons to N_2 and thus, via various intermediate steps, produces ammonia (NH_3). Some of the reducing equivalents are transferred in a side-reaction to H^+. In addition to NH_3, hydrogen is therefore always produced as well.

B. Amino acid biosynthesis: overview ◑

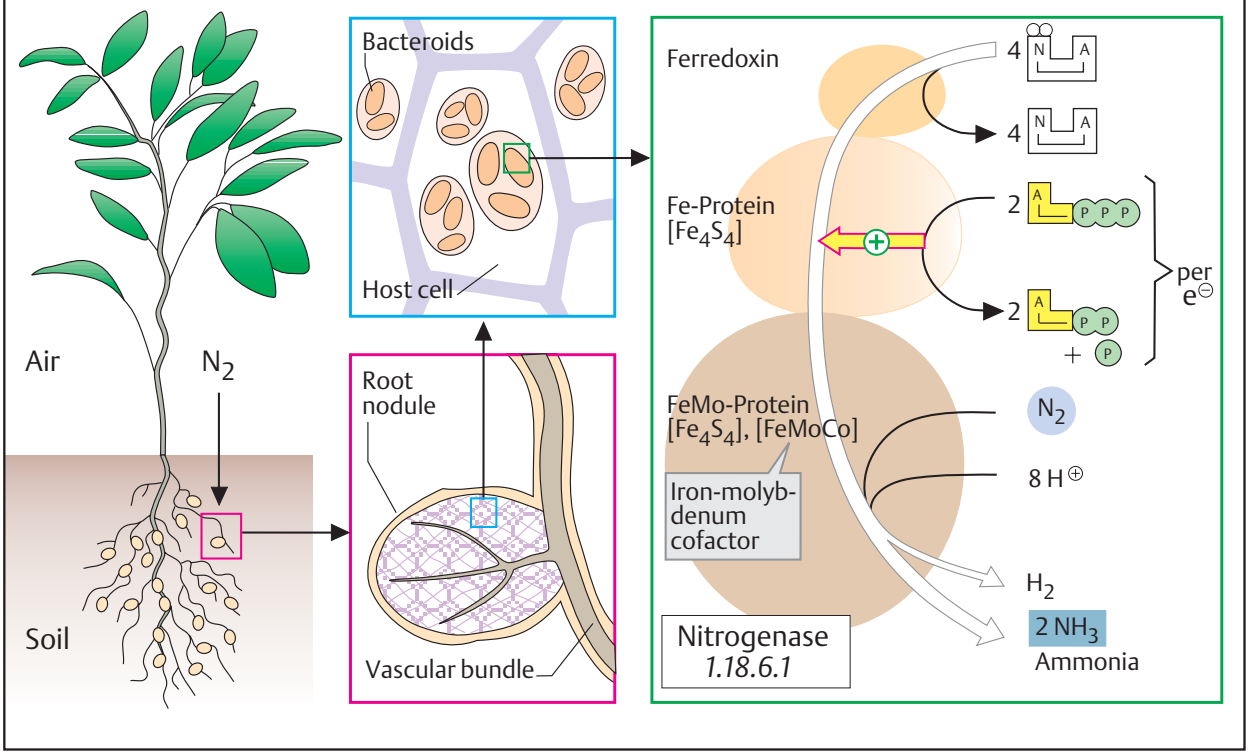
The proteinogenic amino acids (see p. 60) can be divided into **five families** in relation to their biosynthesis. The members of each family are derived from common precursors, which are all produced in the tricarboxylic acid cycle or in catabolic carbohydrate metabolism. An overview of the biosynthetic pathways is shown here; further details are given on pp. 412 and 413.

Plants and microorganisms are able to synthesize all of the amino acids from scratch, but during the course of evolution, mammals have lost the ability to synthesize approximately half of the 20 proteinogenic amino acids. These **essential amino acids** therefore have to be supplied in food. For example, animal metabolism is no longer capable of carrying out de-novo synthesis of the **aromatic amino acids** (tyrosine is only non-essential because it can be formed from phenylalanine when there is an adequate supply available). The **branched-chain amino acids** (valine, leucine, isoleucine, and threonine) as well as **methionine** and **lysine**, also belong to the essential amino acids. Histidine and arginine are essential in rats; whether the same applies in humans is still a matter of debate. A supply of these amino acids in food appears to be essential at least during growth.

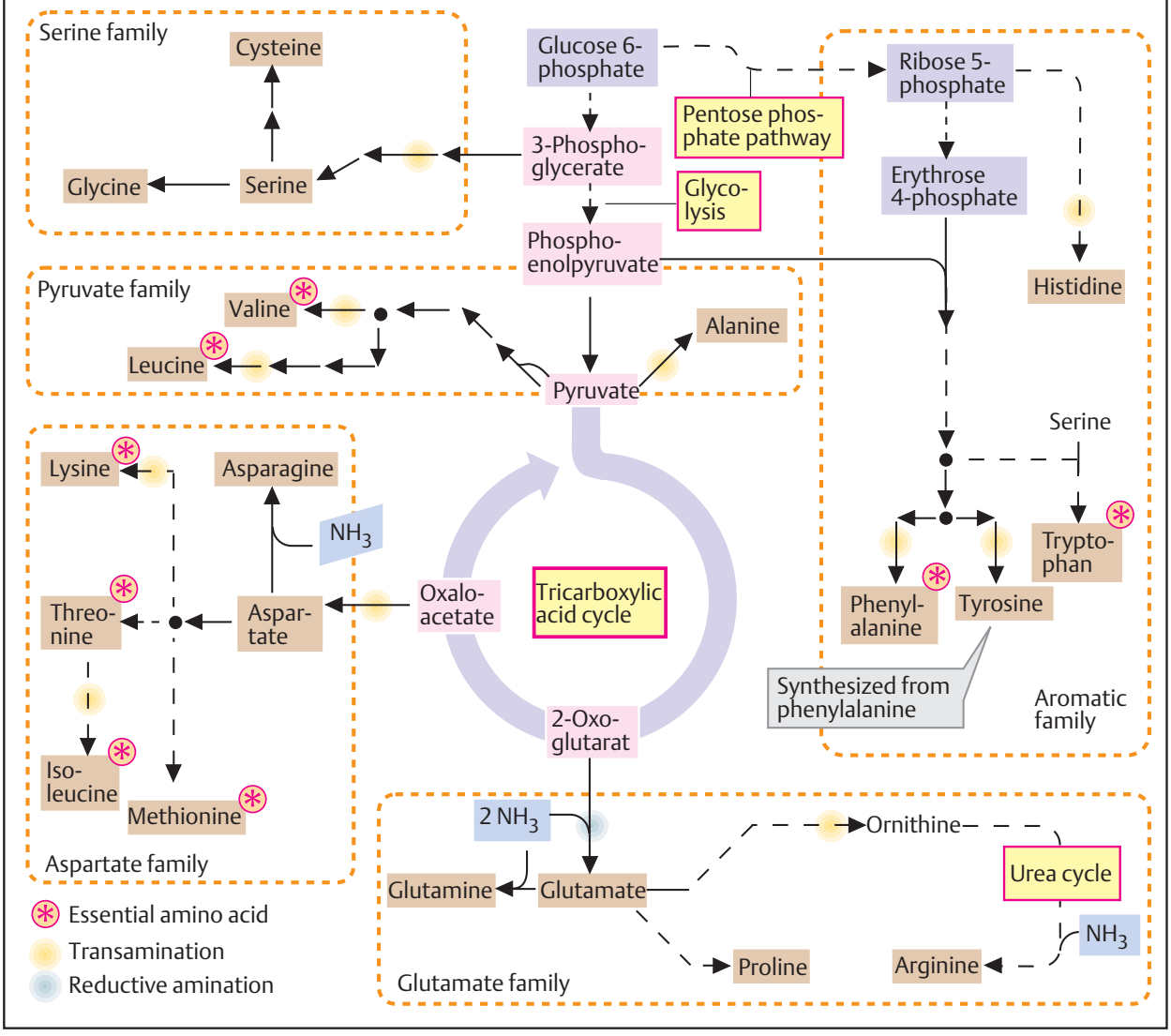
The nutritional value of proteins (see p. 360) is decisively dependent on their essential amino acid content. Vegetable proteins—e.g., those from cereals—are low in lysine and methionine, while animal proteins contain all the amino acids in balanced proportions. As mentioned earlier, however, there are also plants that provide high-value protein. These include the soy bean, one of the plants that is supplied with NH_3 by symbiotic N_2 fixers (**A**).

Non-essential amino acids are those that arise by transamination from 2-oxoacids in the intermediary metabolism. These belong to the **glutamate family** (Glu, Gln, Pro, Arg, derived from 2-oxoglutarate), the **aspartate family** (only Asp and Asn in this group, derived from oxaloacetate), and **alanine**, which can be formed by transamination from pyruvate. The amino acids in the **serine family** (Ser, Gly, Cys) and **histidine**, which arise from intermediates of glycolysis, can also be synthesized by the human body.

A. Symbiotic nitrogen fixation



B. Amino acid biosynthesis: overview



Nucleotide degradation

The nucleotides are among the most complex metabolites. Nucleotide biosynthesis is elaborate and requires a high energy input (see p. 188). Understandably, therefore, bases and nucleotides are not completely degraded, but instead mostly recycled. This is particularly true of the purine bases adenine and guanine. In the animal organism, some 90% of these bases are converted back into nucleoside monophosphates by linkage with phosphoribosyl diphosphate (PRPP) (enzymes [1] and [2]). The proportion of pyrimidine bases that are recycled is much smaller.

A. Degradation of nucleotides ◑

The principles underlying the degradation of purines (**1**) and pyrimidines (**2**) differ. In the human organism, purines are degraded into uric acid and excreted in this form. The purine ring remains intact in this process. In contrast, the ring of the pyrimidine bases (uracil, thymine, and cytosine) is broken down into small fragments, which can be returned to the metabolism or excreted (for further details, see p. 419).

Purine (left). The purine nucleotide **guanosine monophosphate** (**GMP**, **1**) is degraded in two steps—first to the *guanosine* and then to *guanine* (Gua). Guanine is converted by deamination into another purine base, *xanthine*.

In the most important degradative pathway for **adenosine monophosphate** (**AMP**), it is the nucleotide that deaminated, and *inosine monophosphate* (IMP) arises. In the same way as in GMP, the purine base *hypoxanthine* is released from IMP. A single enzyme, *xanthine oxidase* [3], then both converts hypoxanthine into xanthine and xanthine into **uric acid**. An oxo group is introduced into the substrate in each of these reaction steps. The oxo group is derived from *molecular oxygen;* another reaction product is *hydrogen peroxide* (H_2O_2), which is toxic and has to be removed by peroxidases.

Almost all mammals carry out further degradation of uric acid with the help of *uricase*, with further opening of the ring to **allantoin**, which is then excreted. However, the primates, including humans, are not capable of synthesizing allantoin. *Uric acid* is therefore the form of the purines excreted in these species. The same applies to birds and many reptiles. Most other animals continue purine degradation to reach allantoic acid or urea and glyoxylate.

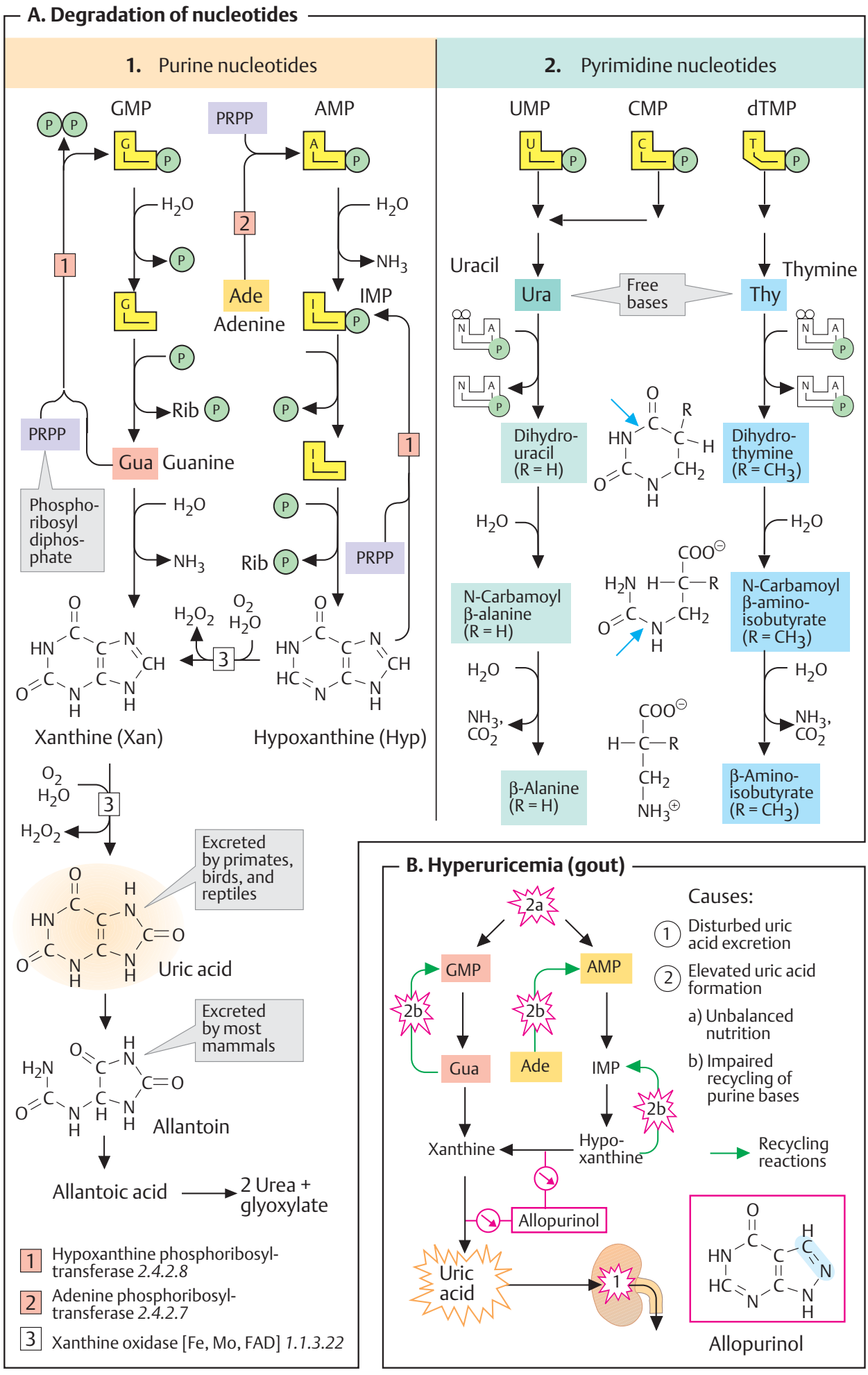
Pyrimidine (right). In the degradation of pyrimidine nucleotides (**2**), the free bases *uracil* (Ura) and *thymine* (Thy) are initially released as important intermediates. Both are further metabolized in similar ways. The pyrimidine ring is first reduced and then hydrolytically cleaved. In the next step, *β-alanine* arises by cleavage of CO_2 and NH_3 as the degradation product of uracil. When there is further degradation, *β-alanine* is broken down to yield acetate, CO_2, and NH_3. Propionate, CO_2, and NH_3 arise in a similar way from *γ-aminoisobutyrate,* the degradation product of thymine (see p. 419).

B. Hyperuricemia ○

The fact that purine degradation in humans already stops at the uric acid stage can lead to problems, since—in contrast to allantoin—uric acid is *poorly soluble in water*. When large amounts of uric acid are formed or uric acid processing is disturbed, excessive concentrations of uric acid can develop in the blood (*hyperuricemia*). This can result in the accumulation of uric acid crystals in the body. Deposition of these crystals in the joints can cause very painful attacks of **gout.**

Most cases of hyperuricemia are due to disturbed uric acid excretion via the kidneys (1). A high-purine diet (e.g., meat) may also have unfavorable effects (2). A rare hereditary disease, *Lesch–Nyhan syndrome,* results from a defect in *hypoxanthine phosphoribosyltransferase* (A, enzyme [1]). The impaired recycling of the purine bases caused by this leads to hyperuricemia and severe neurological disorders.

Hyperuricemia can be treated with *allopurinol,* a competitive inhibitor of xanthine oxidase. This substrate analogue differs from the substrate hypoxanthine only in the arrangement of the atoms in the 5-ring.



Purine and pyrimidine biosynthesis

The bases occurring in nucleic acids are derivatives of the aromatic heterocyclic compounds *purine* and *pyrimidine* (see p. 80). The biosynthesis of these molecules is complex, but is vital for almost all cells. The synthesis of the nucleobases is illustrated here schematically. Complete reaction schemes are given on pp. 417 and 418.

A. Components of nucleobases ○

The **pyrimidine ring** is made up of three components: the nitrogen atom N-1 and carbons C-4 to C-6 are derived from *aspartate,* carbon C-2 comes from HCO_3^-, and the second nitrogen (N-3) is taken from the amide group of *glutamine*.

The synthesis of the **purine ring** is more complex. The only major component is *glycine*, which donates C-4 and C-5, as well as N-7. All of the other atoms in the ring are incorporated individually. C-6 comes from HCO_3^-. Amide groups from *glutamine* provide the atoms N-3 and N-9. The amino group donor for the inclusion of N-1 is *aspartate,* which is converted into fumarate in the process, in the same way as in the urea cycle (see p. 182). Finally, the carbon atoms C-2 and C-8 are derived from formyl groups in N^{10}-formyl-tetrahydrofolate (see p. 108).

B. Pyrimidine and purine synthesis ○

The major intermediates in the biosynthesis of nucleic acid components are the mononucleotides *uridine monophosphate* (UMP) in the pyrimidine series and *inosine monophosphate* (IMP, base: hypoxanthine) in the purines. The synthetic pathways for pyrimidines and purines are fundamentally different. For the pyrimidines, the pyrimidine ring is first constructed and then linked to ribose 5′-phosphate to form a nucleotide. By contrast, synthesis of the purines starts directly from ribose 5′-phosphate. The ring is then built up step by step on this carrier molecule.

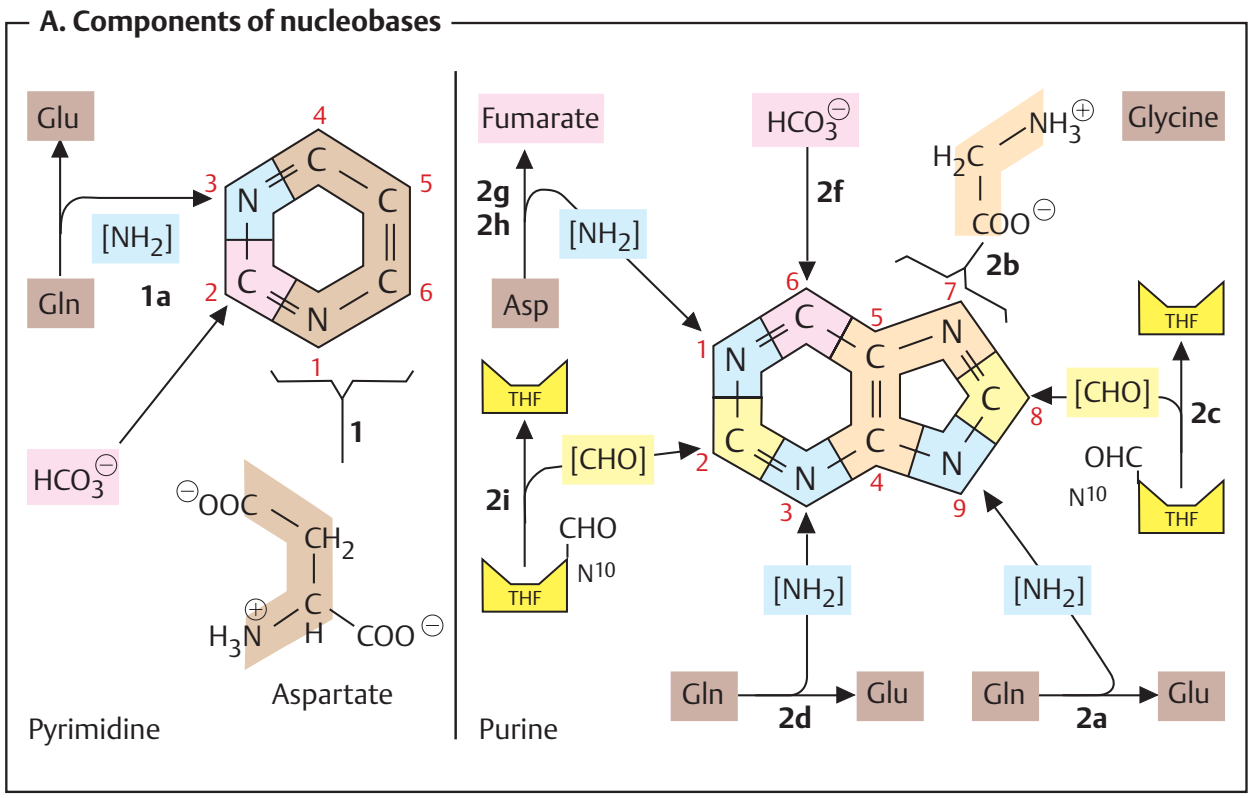
The precursors for the synthesis of the pyrimidine ring are **carbamoyl phosphate**, which arises from glutamate and HCO_3^- (**1a**) and the amino acid **aspartate.** These two components are linked to *N*-**carbamoyl aspartate** (**1b**) and then converted into **dihydroorotate** by closure of the ring (**1c**). In mammals, steps 1a to 1c take place in the cytoplasm, and are catalyzed by a single multifunctional enzyme. In the next step (**1d**), dihydroorotate is oxidized to **orotate** by an FMN-dependent dehydrogenase. Orotate is then linked with **phosphoribosyl diphosphate** (PRPP) to form the nucleotide **orotidine 5′-monophosphate (OMP)**. Finally, decarboxylation yields **uridine 5′-monophosphate (UMP)**.

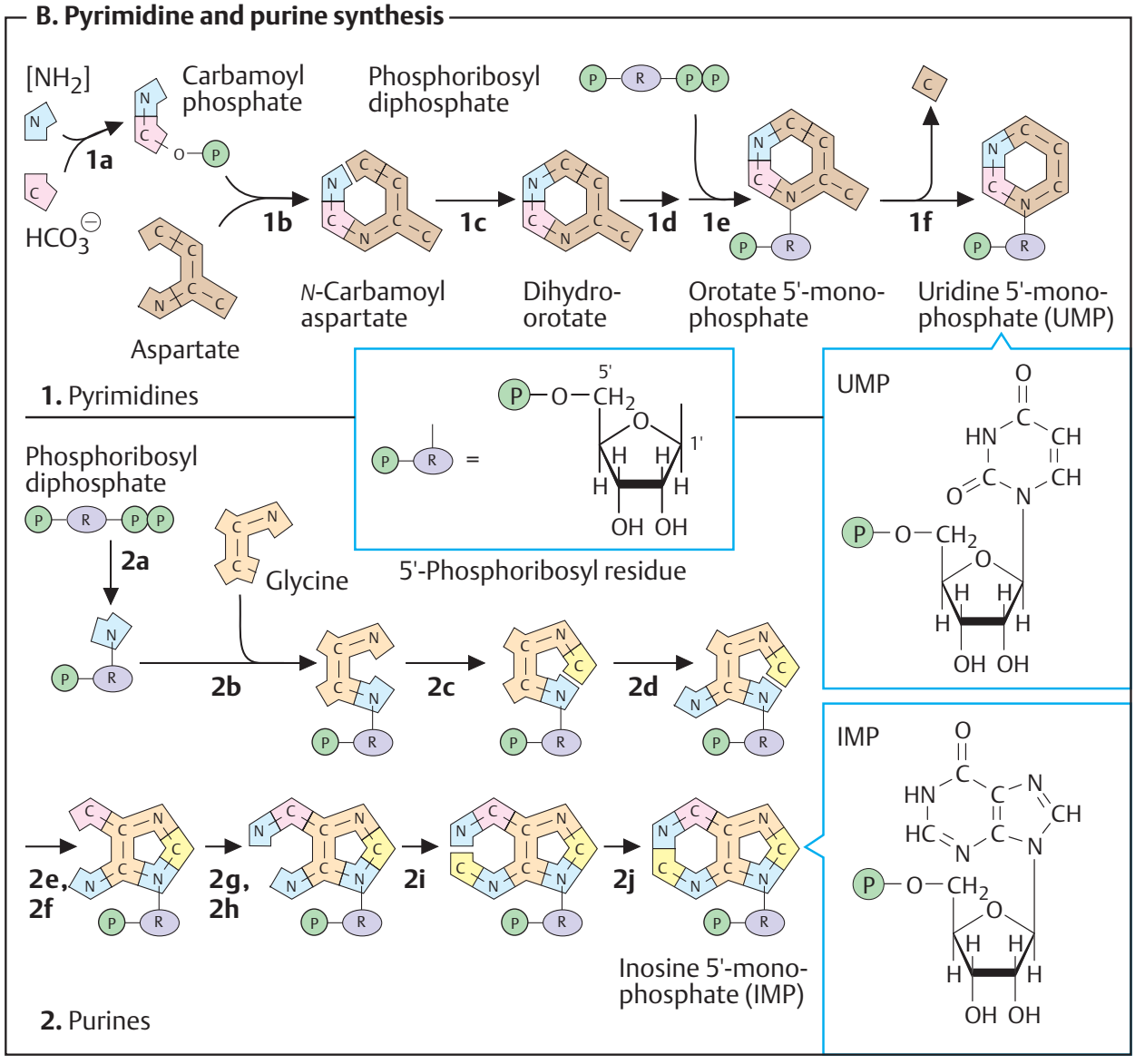
Purine biosynthesis starts with PRPP (the names of the individual intermediates are given on p. 417). Formation of the ring starts with transfer of an amino group, from which the later N-9 is derived (**2a**). Glycine and a formyl group from N^{10}-formyl-THF then supply the remaining atoms of the five-membered ring (**2b**, **2c**). Before the five-membered ring is closed (in step **2f**), atoms N-3 and C-6 of the later six-membered ring are attached (**2d**, **2e**). Synthesis of the ring then continues with N-1 and C-2 (**2g**, **2i**). In the final step (**2j**), the six-membered ring is closed, and **inosine 5′-monophosphate** arises. However, the IMP formed does not accumulate, but is rapidly converted into AMP and GMP. These reactions and the synthesis of the other nucleotides are discussed on p. 190.

Further information

The regulation of bacterial *aspartate carbamoyltransferase* by ATP and CTP has been particularly well studied, and is discussed on p. 116. In animals, in contrast to prokaryotes, it is not ACTase but *carbamoylphosphate synthase* that is the key enzyme in pyrimidine synthesis. It is activated by ATP and PRPP and inhibited by UTP.

The biosynthesis of the purines is also regulated by *feedback inhibition.* ADP and GDP inhibit the formation of PRRPP from ribose-5′-phosphate. Similarly, step **2a** is inhibited by AMP and GMP.





Nucleotide biosynthesis

De novo synthesis of purines and pyrimidines yields the monophosphates IMP and UMP, respectively (see p. 188). All other nucleotides and deoxynucleotides are synthesized from these two precursors. An overview of the pathways involved is presented here; further details are given on p. 417. Nucleotide synthesis by recycling of bases (the salvage pathway) is discussed on p. 186.

A. Nucleotide synthesis: overview ◑

The synthesis of **purine nucleotides** (**1**) starts from **IMP**. The base it contains, *hypoxanthine*, is converted in two steps each into adenine or guanine. The nucleoside monophosphates **AMP** and **GMP** that are formed are then phosphorylated by *nucleoside phosphate kinases* to yield the diphosphates **ADP** and **GDP**, and these are finally phosphorylated into the triphosphates **ATP and GTP**. The nucleoside triphosphates serve as components for RNA, or function as coenzymes (see p. 106). Conversion of the ribonucleotides into deoxyribonucleotides occurs at the level of the *diphosphates* and is catalyzed by *nucleoside diphosphate reductase* (**B**).

The biosynthetic pathways for the **pyrimidine nucleotides** (**2**) are more complicated. The first product, **UMP**, is phosphorylated first to the diphosphate and then to the triphosphate, **UTP**. *CTP synthase* then converts UTP into **CTP**. Since pyrimidine nucleotides are also reduced to deoxyribonucleotides at the diphosphate level, CTP first has to be hydrolyzed by a *phosphatase* to yield **CDP** before **dCDP and dCTP** can be produced.

The DNA component deoxythymidine triphosphate (**dTTP**) is synthesized from UDP in several steps. The base thymine, which only occurs in DNA (see p. 80), is formed by methylation of **dUMP** at the nucleoside monophosphate level. *Thymidylate synthase* and its helper enzyme *dihydrofolate reductase* are important target enzymes for cytostatic drugs (see p. 402).

B. Ribonucleotide reduction ○

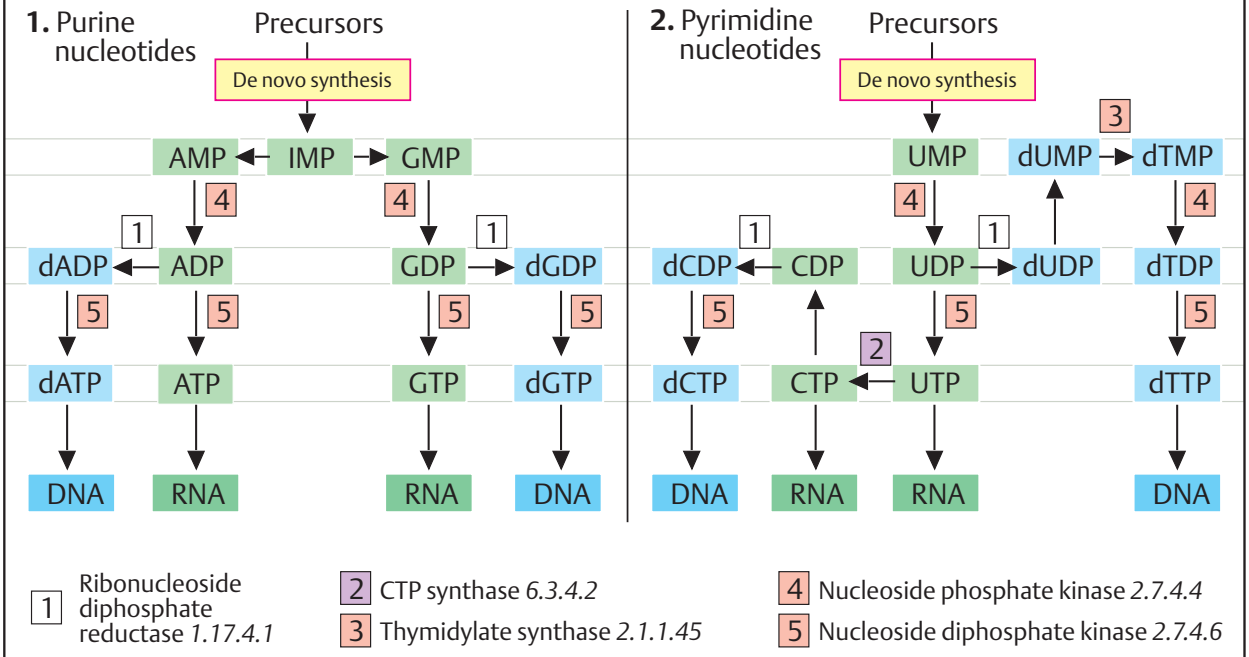
2′-Deoxyribose, a component of DNA, is not synthesized as a free sugar, but arises at the diphosphate level by reduction of ribonucleoside diphosphates. This reduction is a complex process in which several proteins are involved. The reducing equivalents needed come from **NADPH+H$^+$**. However, they are not transferred directly from the coenzyme to the substrate, but first pass through a *redox series* that has several steps (**1**).

In the first step, *thioredoxin reductase* reduces a small redox protein, **thioredoxin**, via enzyme-bound FAD. This involves cleavage of a disulfide bond in thioredoxin. The resulting SH groups in turn reduce a catalytically active disulfide bond in *nucleoside diphosphate reductase* ("ribonucleotide reductase"). The free SH groups formed in this way are the actual electron donors for the reduction of ribonucleotide diphosphates.

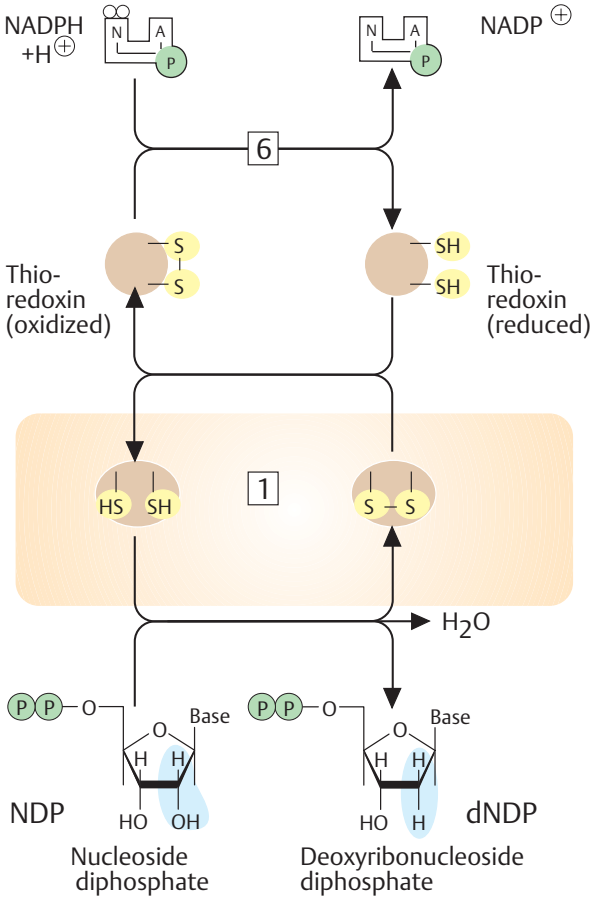
In eukaryotes, ribonucleotide reductase is a tetramer consisting of two R1 and two R2 subunits. In addition to the **disulfide bond** mentioned, a **tyrosine radical** in the enzyme also participates in the reaction (**2**). It initially produces a substrate radical (**3**). This cleaves a water molecule and thereby becomes radical cation. Finally, the deoxyribose residue is produced by reduction, and the tyrosine radical is regenerated.

The regulation of ribonucleotide reductase is complex. The substrate-specificity and activity of the enzyme are controlled by two allosteric binding sites (a and b) in the R1 subunits. ATP and dATP increase or reduce the activity of the reductase by binding at site a. Other nucleotides interact with site b, and thereby alter the enzyme's specificity.

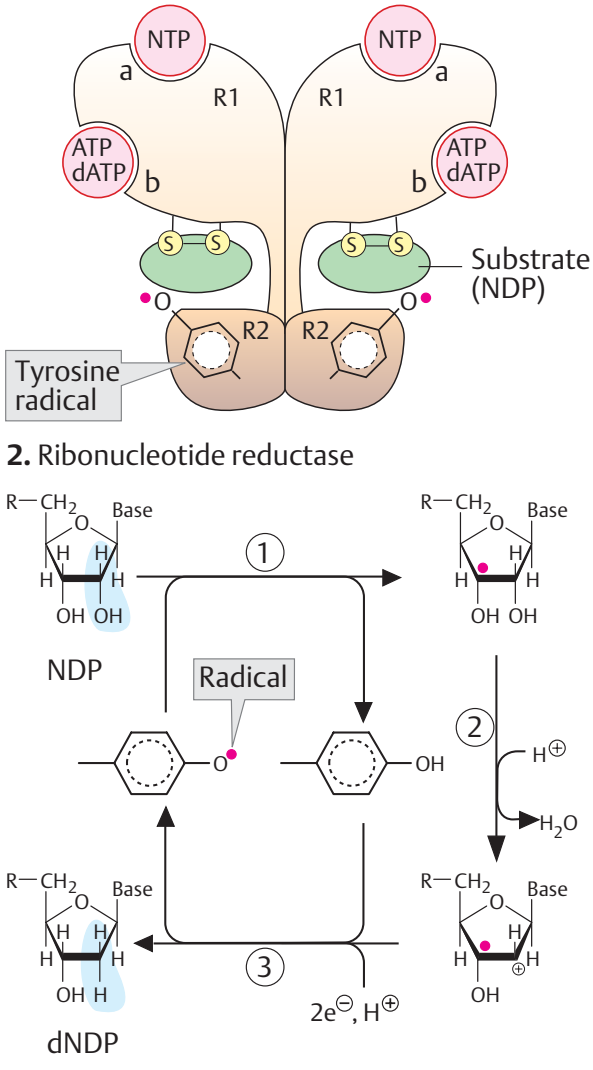
A. Nucleotide synthesis: overview



B. Ribonucleotide reduction



1. Overview



3. Reaction mechanism

Heme biosynthesis

Heme, an iron-containing tetrapyrrole pigment, is a component of O_2-binding proteins (see p. 106) and a coenzyme of various oxidoreductases (see p. 32). Around 85% of heme biosynthesis occurs in the bone marrow, and a much smaller percentage is formed in the liver. Both mitochondria and cytoplasm are involved in heme synthesis.

A. Biosynthesis of heme ○

Synthesis of the tetrapyrrole ring starts in the mitochondria.

[1] **Succinyl CoA** (upper left), an intermediate in the tricarboxylic acid cycle, undergoes condensation with **glycine** and subsequent decarboxylation to yield **5-aminolevulinate** (ALA). The *ALA synthase* responsible for this step is the key enzyme of the whole pathway. Synthesis of ALA synthase is *repressed* and existing enzyme is inhibited by heme, the end product of the pathway. This is a typical example of end-product or *feedback inhibition.*

[2] 5-Aminolevulinate now leaves the mitochondria. In the cytoplasm, two molecules condense to form **porphobilinogen,** a compound that already contains the pyrrole ring. *Porphobilinogen synthase* is inhibited by lead ions. This is why acute lead poisoning is associated with increased concentrations of ALA in the blood and urine.

[3] The tetrapyrrole structure characteristic of the porphyrins is produced in the next steps of the synthetic pathway. *Hydroxymethylbilane synthase* catalyzes the linkage of four porphobilinogen molecules and cleavage of an NH_2 group to yield **uroporphyrinogen III**.

[4] Formation of this intermediate step requires a second enzyme, *uroporphyrinogen III synthase*. If this enzyme is lacking, the "wrong" isomer, uroporphyrinogen I, is formed.

The tetrapyrrole structure of uroporphyrinogen III is still very different from that of heme. For example, the central iron atom is missing, and the ring contains only eight of the 11 double bonds. In addition, the ring system only carries charged R side chains (four acetate and four propionate residues). As heme groups have to act in the apolar interior of proteins, most of the polar side chains have to be converted into less polar groups.

[5] Initially, the four acetate residues (R_1) are decarboxylated into methyl groups. The resulting **coproporphyrinogen III** returns to the mitochondria again. The subsequent steps are catalyzed by enzymes located either on or inside the *inner mitochondrial membrane.*

[6] An *oxidase* first converts two of the propionate groups (R_2) into vinyl residues. The formation of **protoporphyrinogen IX** completes the modification of the side chains.

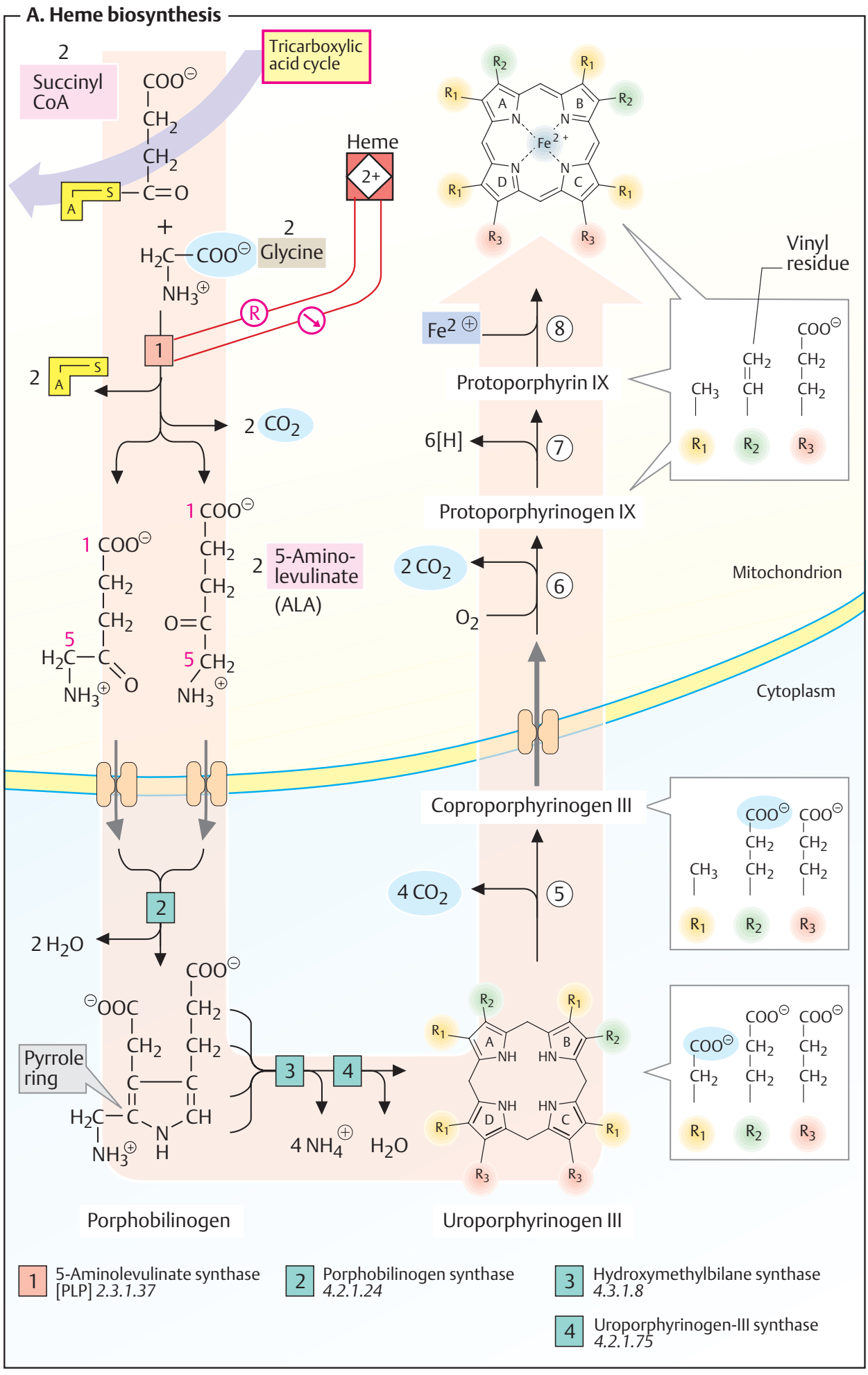
[7] In the next step, another oxidation produces the conjugated π-electron system of **protoporphyrin IX**.

[8] Finally, a divalent iron is incorporated into the ring. This step also requires a specific enzyme, *ferrochelatase.* The **heme b** or **Fe-protoporphyrin IX** formed in this way is found in hemoglobin and myoglobin, for example (see p. 280), where it is noncovalently bound, and also in various oxidoreductases (see p. 106).

Further information

There are a large number of hereditary or acquired disturbances of porphyrin synthesis, known as **porphyrias**, some of which can cause severe clinical pictures. Several of these diseases lead to the excretion of heme precursors in feces or urine, giving them a dark red color. Accumulation of porphyrins in the skin can also occur, and exposure to light then causes disfiguring, poorly healing blisters. Neurological disturbances are also common in the porphyrias.

It is possible that the medieval legends about human vampires ("Dracula") originated in the behavior of porphyria sufferers (avoidance of light, behavioral disturbances, and drinking of blood in order to obtain heme—which markedly improves some forms of porphyria).



Heme degradation

A. Degradation of heme groups ○

Heme is mainly found in the human organism as a prosthetic group in erythrocyte hemoglobin. Around 100–200 million aged erythrocytes per hour are broken down in the human organism. The degradation process starts in reticuloendothelial cells in the spleen, liver, and bone marrow.

[1] After the protein part (globin) has been removed, the tetrapyrrole ring of heme is oxidatively cleaved between rings A and B by *heme oxygenase.* This reaction requires molecular oxygen and $NADPH+H^+$, and produces green **biliverdin**, as well as CO (carbon monoxide) and Fe^{2+}, which remains available for further use (see p. 286).

[2] In another redox reaction, biliverdin is reduced by *biliverdin reductase* to the orange-colored **bilirubin.** The color change from purple to green to yellow can be easily observed in vivo in a bruise or hematoma.

The color of heme and the other *porphyrin systems* (see p. 106) results from their numerous conjugated double bonds. Heme contains a cyclic conjugation (highlighted in pink) that is removed by reaction [1]. Reaction [2] breaks the π system down into two smaller separate systems (highlighted in yellow).

For further degradation, bilirubin is transported to the liver via the blood. As bilirubin is poorly soluble, it is bound to **albumin** for transport**.** Some drugs that also bind to albumin can lead to an increase in free bilirubin.

[3] The hepatocytes take up bilirubin from the blood and conjugate it in the endoplasmic reticulum with the help of **UDP-glucuronic acid** into the more easily soluble **bilirubin monoglucuronides** and **diglucuronides**. To do this, *UDP-glucuronosyltransferase* forms ester-type bonds between the OH group at C-1 of glucuronic acid and the carboxyl groups in bilirubin (see p. 316). The glucuronides are then excreted by active transport into the **bile**, where they form what are known as the **bile pigments**.

Glucuronide synthesis is the rate-determining step in hepatic bilirubin metabolism. Drugs such as *phenobarbital,* for example, can induce both conjugate formation and the transport process.

Some of the bilirubin conjugates are broken down further in the intestine by bacterial *β-glucuronidases.* The bilirubin released is then reduced further via intermediate steps into colorless **stercobilinogen**, some of which is oxidized again into orange to yellow-colored stercobilin. The end products of bile pigment metabolism in the intestine are mostly excreted in feces, but a small proportion is resorbed *(enterohepatic circulation;* see p. 314). When high levels of heme degradation are taking place, stercobilinogen appears as **urobilinogen** in the urine, where oxidative processes darken it to form **urobilin**.

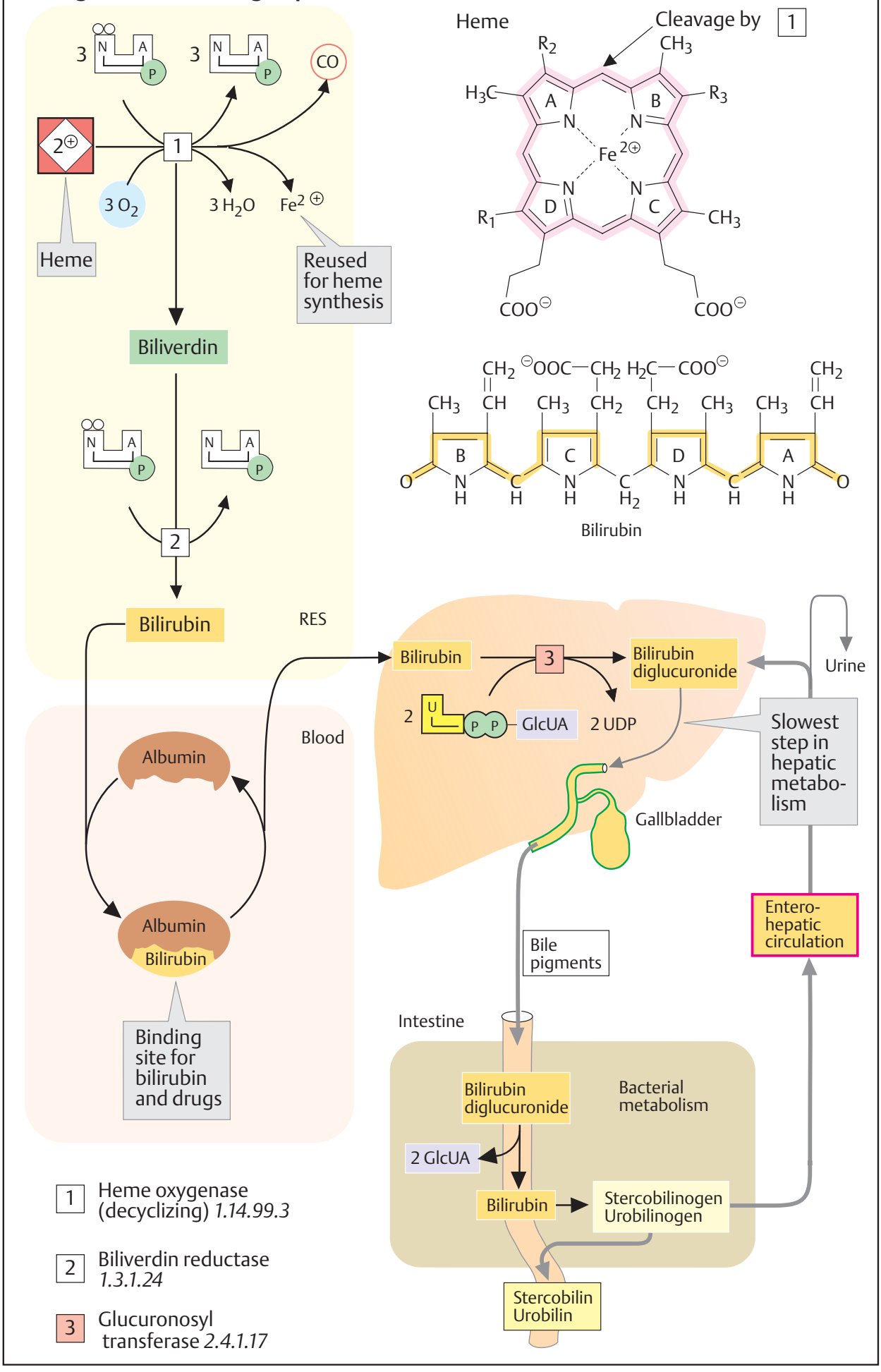
In addition to hemoglobin, other *heme proteins* (myoglobin, cytochromes, catalases, and peroxidases; see p. 32) also supply heme groups that are degraded via the same pathway. However, these contribute only about 10–15% to a total of ca. 250 mg of bile pigment formed per day.

Further information

Hyperbilirubinemias. An elevated bilirubin level (> 10 mg L^{-1}) is known as *hyperbilirubinemia.* When this is present, bilirubin diffuses from the blood into peripheral tissue and gives it a yellow color (jaundice). The easiest way of observing this is in the white conjunctiva of the eyes.

Jaundice can have various causes. If increased erythrocyte degradation (hemolysis) produces more bilirubin, it causes *hemolytic jaundice.* If bilirubin conjugation in the liver is impaired—e.g., due to hepatitis or liver cirrhosis—it leads to *hepatocellular jaundice,* which is associated with an increase in unconjugated (*"indirect"*) bilirubin in the blood. By contrast, if there is a disturbance of bile drainage (*obstructive jaundice,* due to gallstones or pancreatic tumors), then conjugated (*"direct"*) bilirubin in the blood increases. *Neonatal jaundice* (physiologic jaundice) usually resolves after a few days by itself. In severe cases, however, unconjugated bilirubin can cross the blood–brain barrier and lead to brain damage (*kernicterus*).

A. Degradation of heme groups



Structure of cells

A. Comparison of prokaryotes and eukaryotes ●

Present-day living organisms can be divided into two large groups—the prokaryotes and eukaryotes. The **prokaryotes** are represented by bacteria *(eubacteria* and *archaebacteria).* These are almost all small unicellular organisms only a few microns (10^{-6} m) in size. The **eukaryotes** include *fungi*, *plants*, and *animals* and comprise both unicellular and multicellular organisms. Multicellular eukaryotes are made up of a wide variety of cell types that are specialized for different tasks. Eukaryotic cells are much larger than prokaryotic ones (volume ratio approximately 2000 : 1). The most important distinguishing feature of these cells in comparison with the prokaryotes is the fact that they have a **nucleus** (*karyon* in Greek—hence the term).

In comparison with the prokaryotes, eukaryotic cells have greater specialization and complexity in their structure and functioning. Eukaryotic cells are structured into *compartments* (see below). The metabolism and synthesis of macromolecules are distributed through these reaction spaces and are separately regulated. In prokaryotes, these functions are organized in a simpler fashion and are spatially closely related.

Although the storage and transfer of genetic information function according to the same principle in the prokaryotes and eukaryotes, there are also differences. Eukaryotic DNA consists of very long, linear molecules with a total of 10^7 to more than 10^{10} base pairs (bp), only a small fraction of which are used for genetic information. In eukaryotes, the genes (20000–50000 per genome) are usually interrupted by non-coding regions (*introns*). Eukaryotic DNA is located in the nucleus, where together with histones and other proteins it forms the chromatin (see p. 238).

In prokaryotes, by contrast, DNA is ring-shaped, much shorter (up to 5 10^6 bp), and located in the cytoplasm. Almost all of it is used for information storage, and it does not contain any introns.

B. Structure of an animal cell ●

In the human body alone, there are at least 200 different cell types. The illustration outlines the basic structures of an animal cell in an extremely simplified way. The details given regarding the proportion of the compartments relative to cell volume (highlighted in yellow) and their numbers per cell frequency (blue) refer to mammalian hepatocytes (liver cells). The figures can vary widely from cell type to cell type.

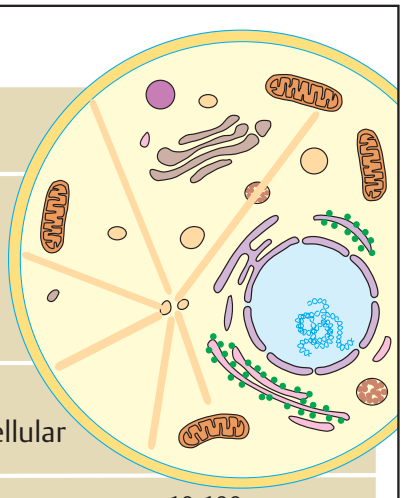
The eukaryotic cell is subdivided by membranes. On the outside, it is enclosed by a **plasma membrane.** Inside the cell, there is a large space containing numerous components in solution—the **cytoplasm**. Additional membranes divide the internal space into *compartments* (confined reaction spaces). Welldefined compartments of this type are known as **organelles**.

The largest organelle is the **nucleus** (see p. 208). It is easily recognized using the light microscope. The **endoplasmic reticulum** (ER), a closed network of shallow sacs and tubules (see pp. 226ff.), is linked with the outer membrane of the nucleus. Another membrane-bound organelle is the **Golgi apparatus** (see p. 228), which resembles a bundle of layered slices. The **endosomes** and **exosomes** are bubble-shaped compartments *(* vesicles*)* that are involved in the exchange of substances between the cell and its surroundings. Probably the most important organelles in the cell's metabolism are the **mitochondria,** which are around the same size as bacteria (see pp. 210ff.). The **lysosomes** and **peroxisomes** are small, globular organelles that carry out specific tasks. The whole cell is traversed by a framework of proteins known as the **cytoskeleton** (see pp. 204ff.).

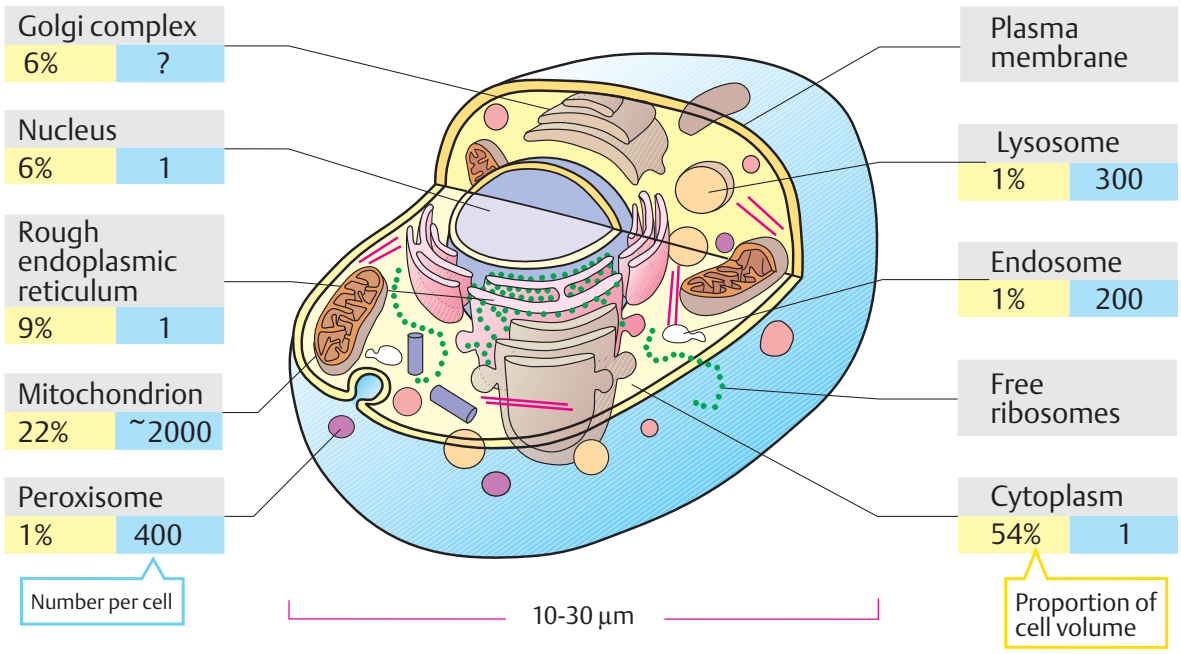
In addition to these organelles, plant cells (see p. 43) also have plastids—eg., **chloroplasts**, in which photosynthesis takes place (see p. 128). In their interior, there is a large, fluid-filled **vacuole**. Like bacteria and fungi, plant cells have a rigid cell wall consisting of polysaccharides and proteins.

A. Comparison of prokaryotes and eukaryotes

Prokaryotes	Eukaryotes
Organisms	
 1-10 µm Eubacteria Archaebacteria	Fungi Plants Animals
Form	
Single-celled	Single or multi-cellular
Organelles, cytoskeleton, cell division apparatus	
Missing	Present, complicated, specialized
DNA	
Small, circular, no introns, plasmids	Large, in nucleus, many introns
RNA: Synthesis and maturation	
Simple, in cytoplasm	Complicated, in nucleus
Protein: Synthesis and maturation	
Simple, coupled with RNA synthesis	Complicated, in the cytoplasm and the rough endoplasmic reticulum
Metabolism	
Anaerobic or aerobic very flexible	Mostly aerobic, compartmented
Endocytosis and Exocytosis	
no	yes



B. Structure of an animal cell



Cell fractionation

A. Isolation of cell organelles ○

To investigate the individual compartments of the cell (see p. 196), various procedures have been developed to enrich and isolate cell organelles. These are mainly based on the size and density of the various organelles.

The isolation of cell components starts with **disruption** of the tissue being examined and subsequent **homogenization** of it (breaking down the cells) in a suitable buffer (see below). Homogenization using the "Potter" (the Potter–Elvehjem homogenizer, a rotating Teflon pestle in a glass cylinder) is particularly suitable for animal tissue. This method is very gentle and is therefore used to isolate fragile structures and molecules. Other cell disruption procedures include **enzymatic lysis** with the help of enzymes that break down the cell wall, **mechanical disruption** by grinding frozen tissue, cutting or smashing with rotating knives, large pressure changes, osmotic shock, and repeated freezing and thawing.

To isolate intact organelles, it is important for the homogenization solution to be *isotonic*—i. e., the osmotic value of the buffer has to be the same as that of the interior of the cell. If hypotonic solutions were used, the organelles would take up water and burst, while in hypertonic solutions they would shrink.

Homogenization is followed by coarse **filtration** through gauze to remove intact cells and connective-tissue fragments. The actual fractionation of cellular components is then carried out by **centrifugation steps**, in which the gravitational force (given as multiples of the earth's gravity, g = 9.81 m s^{-2}) is gradually increased (*differential centrifugation*; see p. 200). Due to the different shapes and densities of the organelles, this leads to successive sedimentation of each type out of the suspension.

Nuclei already sediment at low accelerations that can be achieved with bench-top centrifuges. Decanting the residue (the "supernatant") and carefully suspending the sediment (or "pellet") in an isotonic medium yields a fraction that is enriched with nuclei. However, this fraction may still contain other cellular components as contaminants—e. g., fragments of the cytoskeleton.

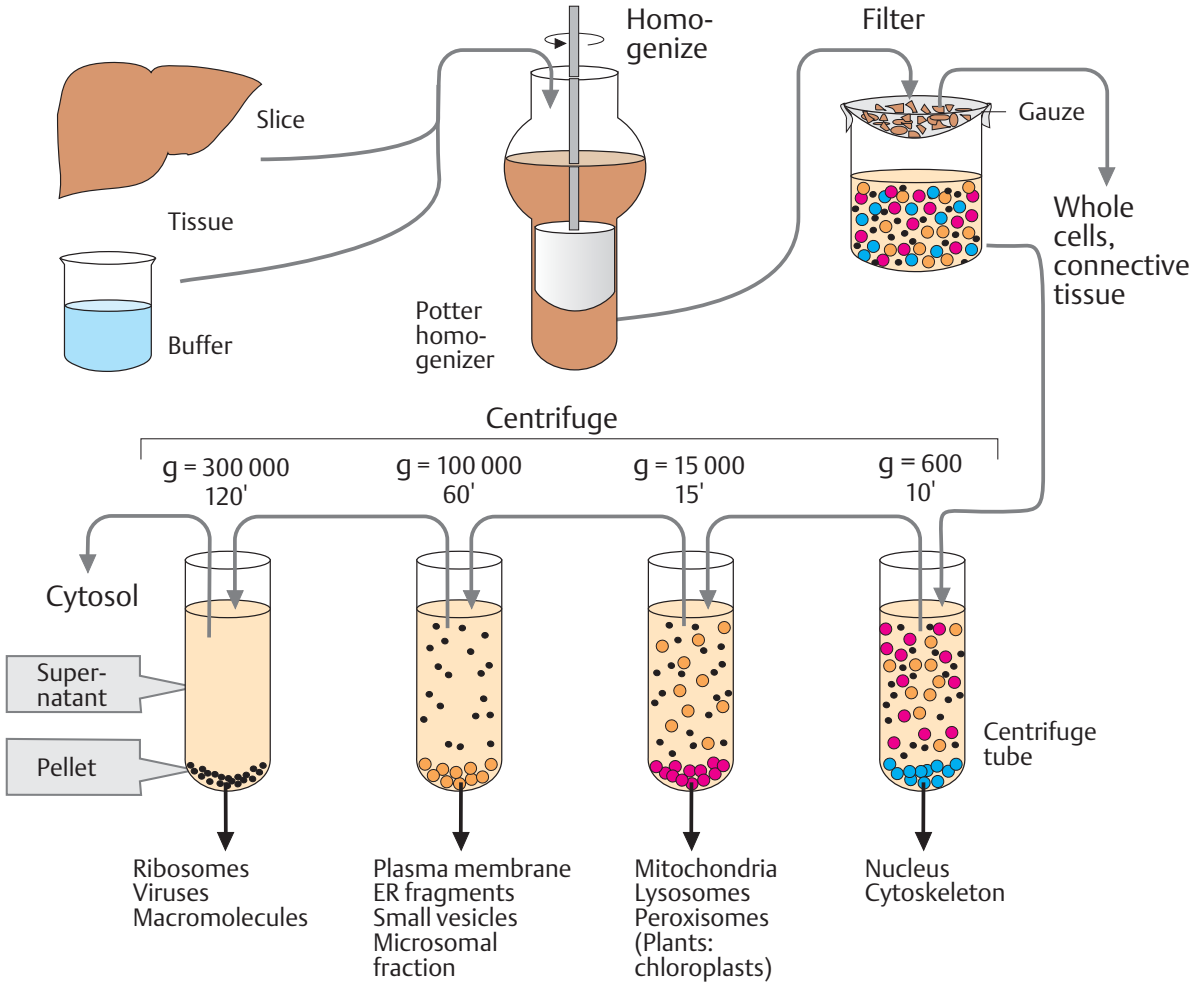
Particles that are smaller and less dense than the nuclei can be obtained by step-by-step acceleration of the gravity on the supernatant left over from the first centrifugation. However, this requires very powerful centrifuges (high-speed centrifuges and ultracentrifuges). The sequence in which the fractions are obtained is: **mitochondria, membrane vesicles**, and **ribosomes**. Finally, the supernatant from the last centrifugation contains the **cytosol** with the cell's soluble components, in addition to the buffer.

The isolation steps are carried out at low temperatures on principle (usually 0–5 °C), to slow down degradation reactions—e. g., due to released enzymes and other influencing factors. The addition of thiols and chelating agents protects functional SH groups from oxidation. Isolated cell organelles quickly lose their biological activity despite these precautions. Nevertheless, it is possible by working carefully to isolate mitochondria that will still take up substrates for a few hours in the test tube and produce ATP via oxidative phosphorylation.

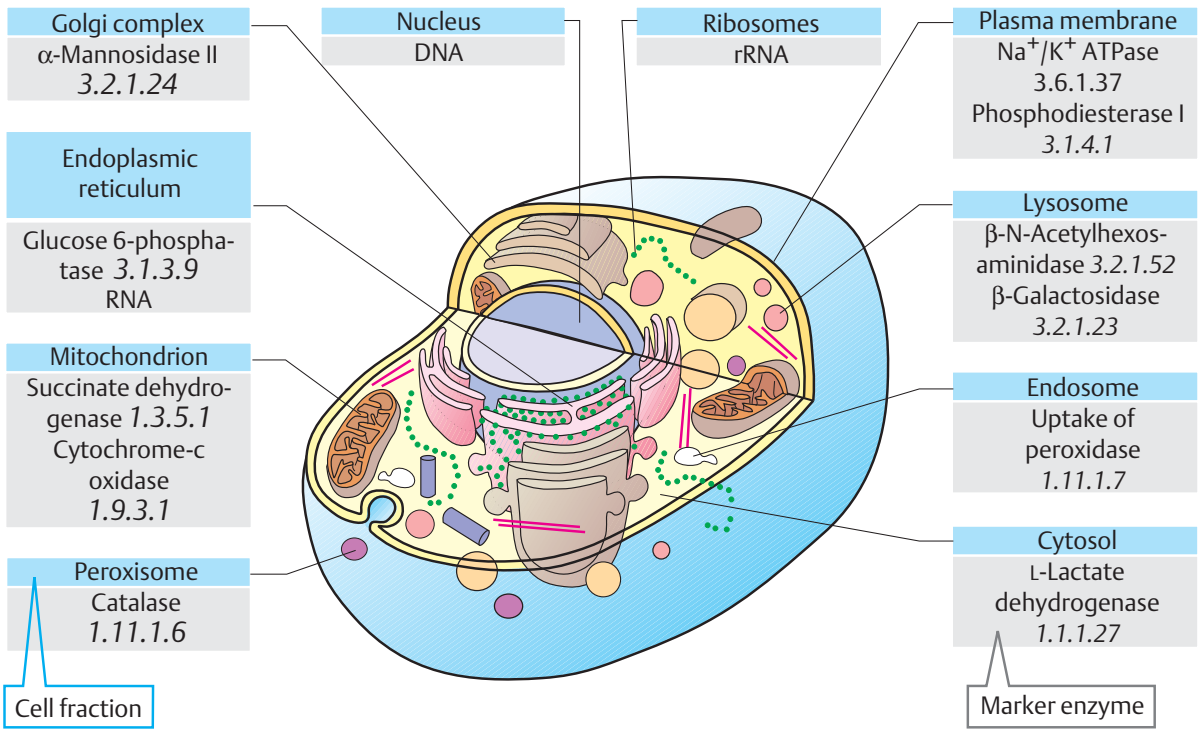
B. Marker molecules ○

During cell fractionation, it is very important to analyze the purity of the fractions obtained. Whether or not the intended organelle is present in a particular fraction, and whether or not the fraction contains other components, can be determined by analyzing characteristic **marker molecules.** These are molecules that occur exclusively or predominantly in one type of organelle. For example, the activity of organelle-specific enzymes **(marker enzymes)** is often assessed. The distribution of marker enzymes in the cell reflects the compartmentation of the processes they catalyze. These reactions are discussed in greater detail here under the specific organelles.

A. Isolation of cell organelles



B. Marker molecules



Centrifugation

A. Principles of centrifugation ○

In a solution, particles whose density is higher than that of the solvent sink (*sediment*), and particles that are lighter than it *float* to the top. The greater the difference in density, the faster they move. If there is no difference in density (isopyknic conditions), the particles *hover*. To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful "centrifugal force" provided by a centrifuge.

Equipment. The acceleration achieved by centrifugation is expressed as a multiple of the earth's gravitational force (g = 9.81 m s^{-2}). Bench-top centrifuges can reach acceleration values of up to 15000 g, while highspeed refrigerated centrifuges can reach 50000 g and ultracentrifuges, which operate with refrigeration and in a vacuum, can reach 500000 g. Two types of rotor are available in high-powered centrifuges: *fixed angle rotors* and *swingout rotors* that have movable bucket containers. The tubes or buckets used for centrifugation are made of plastic and have to be very precisely adjusted to avoid any imbalances that could lead to accidents.

Theory. The velocity (v) of particle sedimentation during centrifugation depends on the angular velocity ω of the rotor, its effective radius (r_{eff}, the distance from the axis of rotation), and the particle's sedimentation properties. These properties are expressed as the **sedimentation coef•cient** S (1 Svedberg, = 10^{-13} s). The sedimentation coef cient depends on the mass M of the particle, its shape (expressed as the coef cient of friction, f), and its density (expressed as the reciprocal density $\bar{v}$, "partial specific volume").

At the top right, the diagram shows the densities and sedimentation coef cients for biomolecules, cell organelles, and viruses. Proteins and protein-rich structures have densities of around 1.3 g cm^{-3}, while nucleic acids show densities of up to 2 g cm^{-3}. Equilibrium sedimentation of nucleic acids therefore requires high-density media—e.g., concentrated solutions of cesium chloride (CsCl). To allow comparison of S values measured in different media, they are usually corrected to values for water at 20 °C ("S_{20w}").

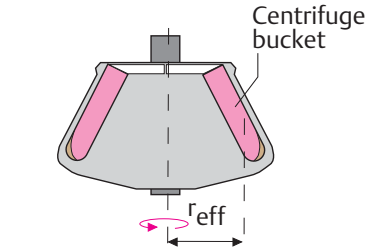
B. Density gradient centrifugation ○

Density gradient centrifugation is used to separate macromolecules that differ only slightly in size or density. Two techniques are commonly used.

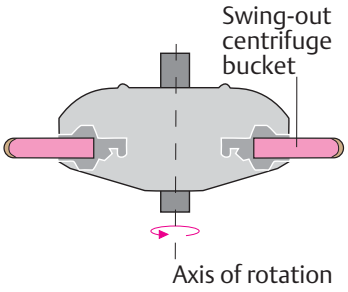
In **zonal centrifugation,** the sample being separated (e.g., a cell extract or cells) is placed on top of the centrifugation solution as a thin layer. During centrifugation, the particles move through the solution due to their greater density. The rate of movement basically depends on their molecular mass (see **A**, formulae). Centrifugation stops before the particles reach the bottom of the tube. Drilling a hole into the centrifugation tube and allowing the contents to drip out makes it possible to collect the different particles in separate fractions. During centrifugation, the solution tube is stabilized in the tube by a **density gradient**. This consists of solutions of carbohydrates or colloidal silica gel, the concentration of which increases from the surface of the tube to the bottom. Density gradients prevent the formation of convection currents, which would impair the separation of the particles.

Isopyknic centrifugation, which takes much longer, starts with a CsCl solution in which the sample material (e.g., DNA, RNA, or viruses) is homogeneously distributed. A density gradient only forms *during* centrifugation, as a result of sedimentation and diffusion processes. Each particle moves to the region corresponding to its own *buoyant density*. Centrifugation stops once equilibrium has been reached. The samples are obtained by fractionation, and their concentration is measured using the appropriate methods.

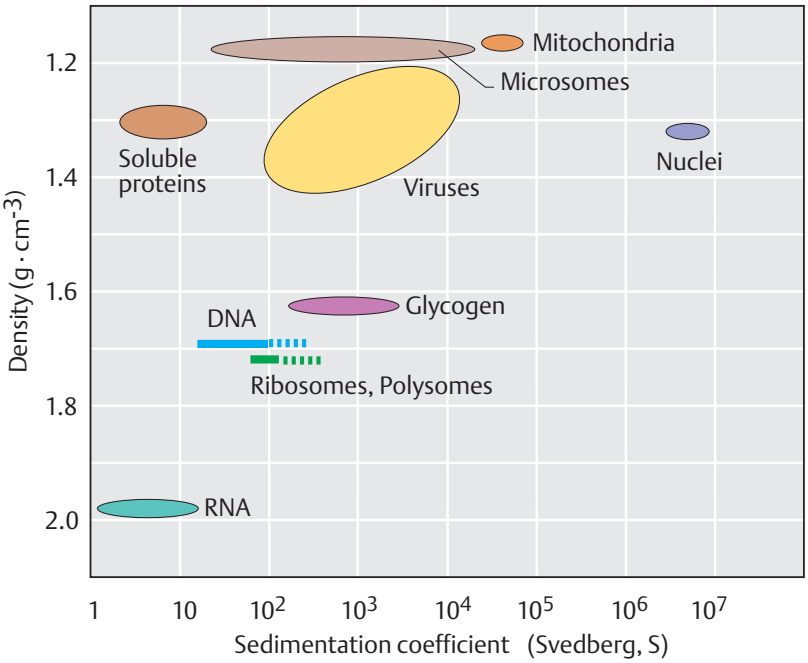
A. Principles of centrifugation



Fixed angle rotor



Swing-out bucket rotor



g: Gravitational acceleration

v: Sedimentation velocity (cm · s^{-1})

ω: Angular velocity (rad · s^{-1})

r_{eff}: Effective radius (cm)

$$g = \omega^2 \cdot r_{eff}$$

$$v = \omega^2 \cdot r_{eff} \cdot s$$

$$s = \frac{M \cdot (1 - \bar{v} \cdot r)}{f}$$

s: Sedimentation coefficient ($S = 10^{-13}$ s)

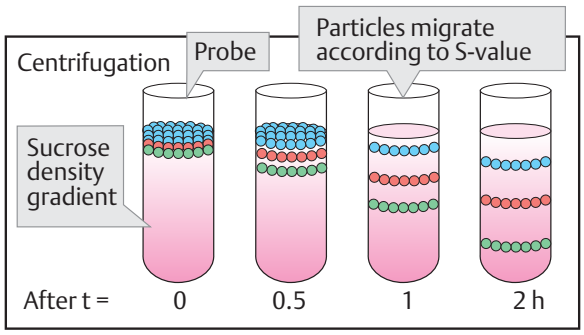
M: Molecular mass

$\bar{v}$: Partial specific particle volume ($cm^3 \cdot g^{-1}$)

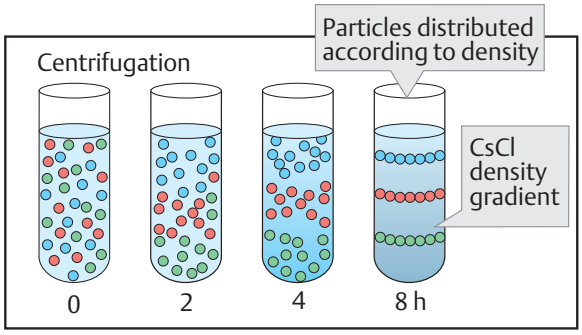
r: Density of the solution ($g \cdot cm^3$)

f: Coefficient of friction

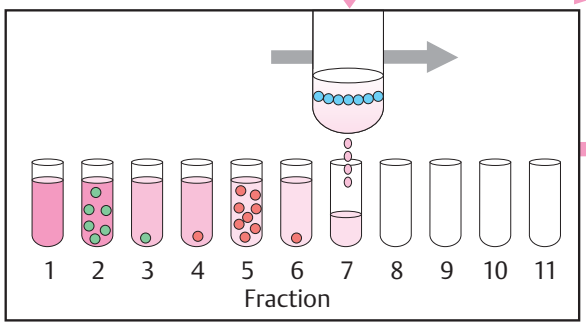
B. Density gradient centrifugation



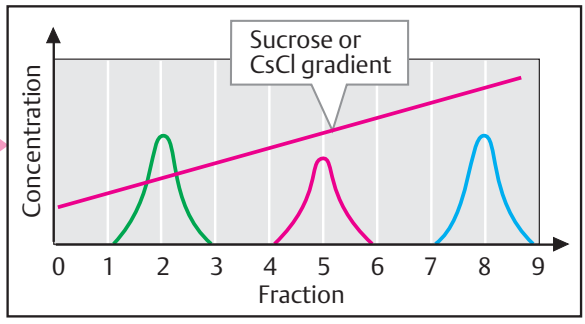
Zonal centrifugation



Isopyknic centrifugation



Fractionation



Detection

Cell components and cytoplasm

The Gram-negative bacterium *Escherichia coli (E. coli)* is a usually harmless symbiont in the intestine of mammals. The structure and characteristics of this organism have been particularly well characterized. *E. coli* is also frequently used in genetic engineering (see p. 258).

A. Components of a bacterial cell ◑

A single *E. coli* cell has a **volume** of about 0.88 μm^3. One-sixth of this consists of membranes and one-sixth is DNA (known as the "nucleoid"). The rest of the internal space of the cell is known as **cytoplasm** (not "cytosol"; see p. 198).

The main component of *E. coli*—as in all cells—is **water** (70%). The other components are **macromolecules** (proteins, nucleic acids, polysaccharides), **small organic molecules**, and **inorganic ions**. The majority of the macromolecules are proteins, which represent ca. 55% of the dry mass of the cell. When a number of assumptions are made about the distribution and size (average mass 40 kDa) of proteins, it can be estimated that there are approximately 250000 protein molecules in the cytoplasm of an *E. coli* cell. In eukaryotic cells, which are about a thousand times larger, it is estimated that the number of protein molecules is in the order of several billion.

B. Looking inside a bacterial cell ○

The illustration shows a schematic view inside the **cytoplasm** of *E. coli*, magnified approximately one million times. At this magnification, a single carbon atom would be the size of a grain of salt, and an ATP molecule would be as large as a grain of rice. The detail shown is 100 nm long, corresponding to about 1/600th of the volume of a cell in *E. coli*. To make the macromolecules clearer, small molecules such as water, cofactors, and metabolites have all been omitted from the illustration. The section of the cytoplasm shown contains:

- Several hundred **macromolecules**, which are needed for protein biosynthesis—i. e., 30 ribosomes, more than 100 protein factors, 30 aminoacyl–tRNA synthases, 340 tRNA molecules, 2–3 mRNAs (each of which is 10 times the length of the section shown), and six molecules of RNA polymerase.
- About 330 other enzyme molecules, including 130 glycolytic enzymes and 100 enzymes from the tricarboxylic acid cycle.
- 30000 **small organic molecules** with masses of 100–1000 Da—e. g., metabolites of the intermediary metabolism and coenzymes. These are shown at a magnification 10 times higher in the bottom right corner.
- And finally, 50000 **inorganic ions.** The rest consists of water.

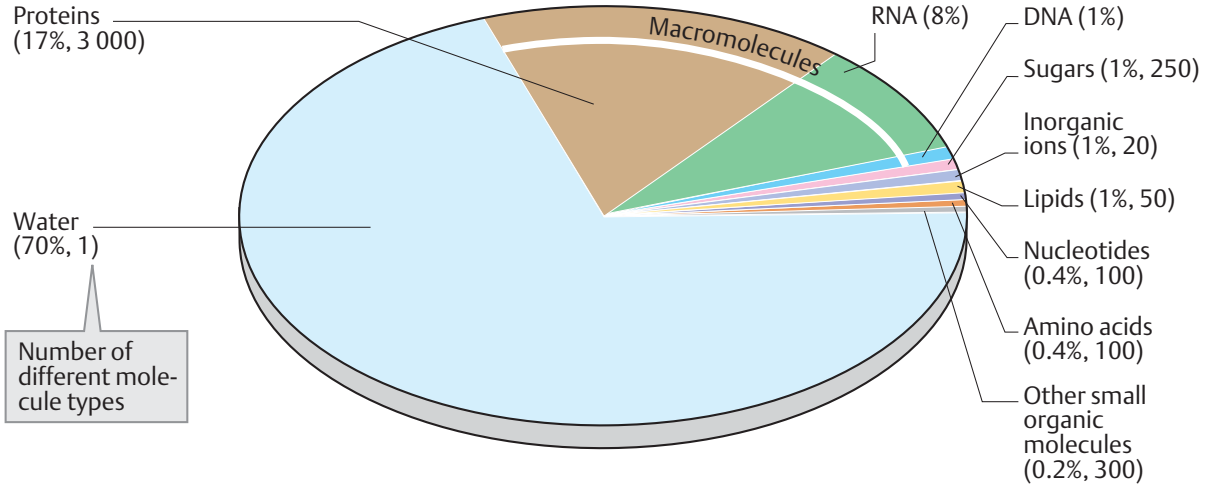
The illustration shows that the cytoplasm of cells is a compartment densely packed with macromolecules and smaller organic molecules. The distances between organic molecules are small. They are only separated by a few water molecules.

All of the molecules are in motion. Due to constant collisions, however, they do not advance in a straight path but move in zigzags. Due to their large mass, proteins are particularly slow. However, they do cover an average of 5 nm in 1 ms—a distance approximately equal to their own length. Statistically, a protein is capable of reaching any point in a bacterial cell in less than a second.

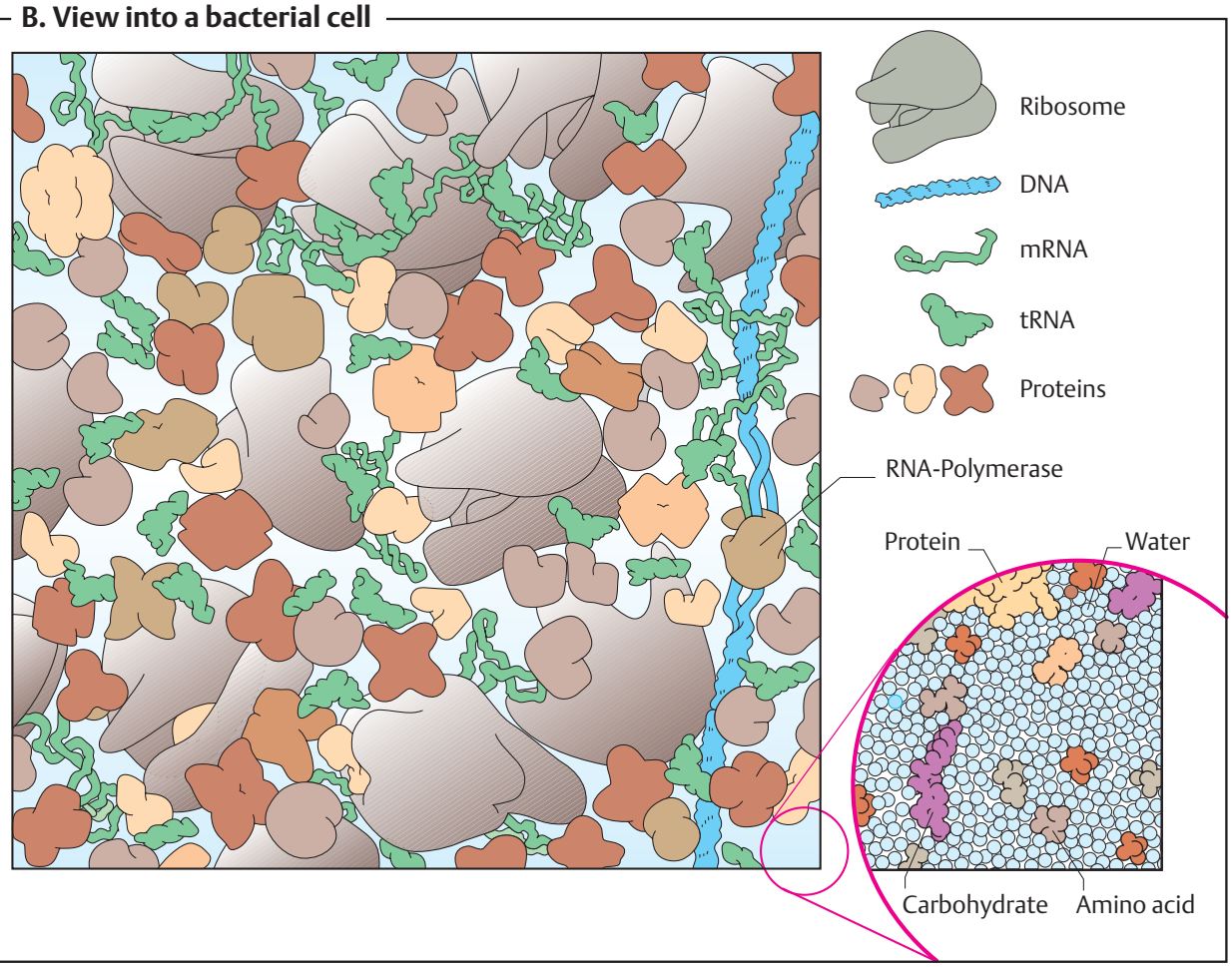
C. Biochemical functions of the cytoplasm ◑

In eukaryotes, the cytoplasm, representing slightly more than 50% of the cell volume, is the most important cellular compartment. It is the *central reaction space of the cell.* This is where many important pathways of the intermediary metabolism take place—e. g., glycolysis, the pentose phosphate pathway, the majority of gluconeogenesis, and fatty acid synthesis. Protein biosynthesis (translation; see p. 250) also takes place in the cytoplasm. By contrast, fatty acid degradation, the tricarboxylic acid cycle, and oxidative phosphorylation are located in the mitochondria (see p. 210).

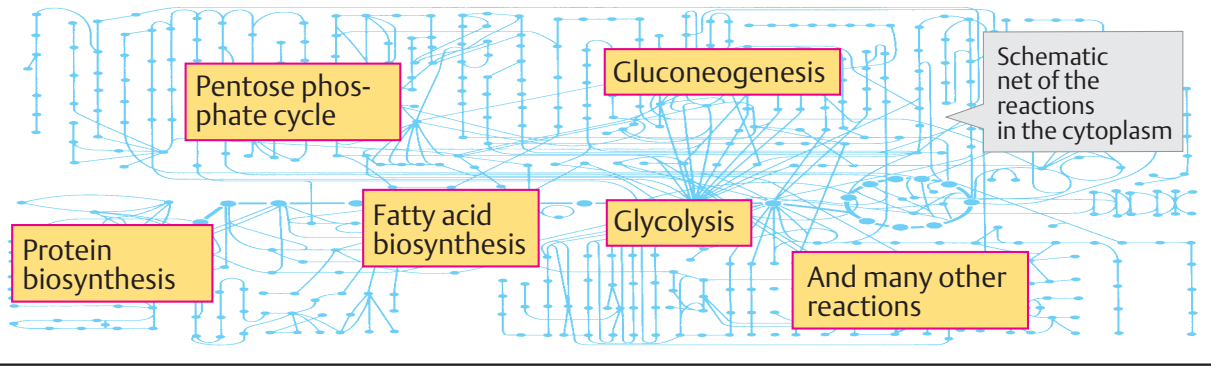
A. Components of a bacterial cell



B. View into a bacterial cell



C. Biochemical functions of the cytoplasm



Cytoskeleton: components

The cytoplasm of eukaryotic cells is traversed by three–dimensional scaffolding structures consisting of filaments (long protein fibers), which together form the **cytoskeleton**. These filaments are divided into three groups, based on their *diameters*: **microfilaments** (6–8 nm), **intermediate filaments** (ca. 10 nm), and **microtubules** (ca. 25 nm). All of these filaments are polymers assembled from protein components.

A. Actin ◑

Actin, the most abundant protein in eukaryotic cells, is the protein component of the **microfilaments** (actin filaments). Actin occurs in two forms—a monomolecular form (**G actin**, globular actin) and a polymer (**F actin**, filamentous actin). G actin is an asymmetrical molecule with a mass of 42 kDa, consisting of two domains. As the ionic strength increases, G actin aggregates reversibly to form F actin, a helical homopolymer. G actin carries a firmly bound ATP molecule that is slowly hydrolyzed in F actin to form ADP. Actin therefore also has enzyme properties (*ATPase* activity).

As individual G actin molecules are always oriented in the same direction relative to one another, F actin consequently has *polarity*. It has two different ends, at which polymerization takes place at different rates. If the ends are not stabilized by special proteins (as in muscle cells), then at a critical concentration of G actin the (+) end of F actin will constantly grow, while the (–) end simultaneously decays. These partial processes can be blocked by fungal toxins experimentally. **Phalloidin**, a toxin contained in the *Amanita phalloides* mushroom, inhibits decay by binding to the (–) end. By contrast, **cytochalasins**, mold toxins with cytostatic effects, block polymerization by binding to the (+) end.

Actin–associated proteins. The cytoplasm contains more than 50 different proteins that bind specifically to G actin and F actin. Their actin uptake has various different functions. This type of bonding can serve to regulate the G actin pool (example: *profilin*), influence the polymerization rate of G actin (*villin*), stabilize the chain ends of F actin (*fragin, γ–actinin*), attach filaments to one another or to other cell components (*villin, α–actinin, spectrin),* or disrupt the helical structure of F actin (*gelsolin*). The activity of these proteins is regulated by protein kinases via Ca^{2+} and other second messengers (see p. 386).

B. Intermediate filaments ◑

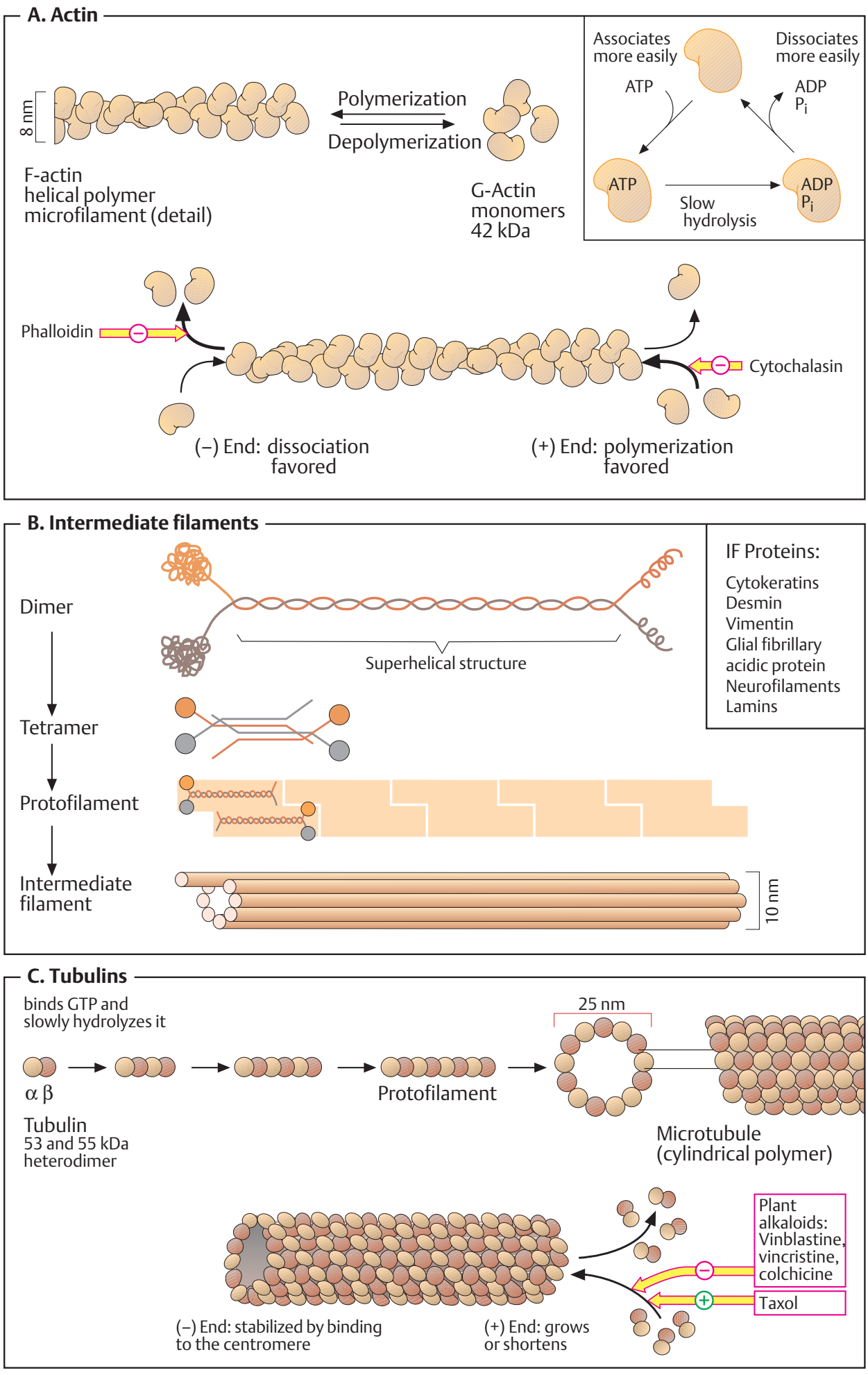
The components of the intermediate filaments belong to five related protein families. They are specific for particular cell types. Typical representatives include the *cytokeratins, desmin, vimentin, glial fibrillary acidic protein* (GFAP), and *neurofilament*. These proteins all have a rod–shaped basic structure in the center, which is known as a *superhelix* ("coiled coil"; see keratin, p. 70). The dimers are arranged in an antiparallel fashion to form tetramers. A staggered head-to–head arrangement produces **protofilaments**. Eight protofilaments ultimately form an intermediary filament.

Free protein monomers of intermediate filaments rarely occur in the cytoplasm, in contrast to microfilaments and microtubules. Their polymerization leads to stable polymers that have no polarity.

C. Tubulins ◑

The basic components of the tube-shaped **microtubules** are α– and β–**tubulin** (53 and 55 kDa). These form α,β-heterodimers, which in turn polymerize to form linear protofilaments. Thirteen protofilaments form a ring-shaped complex, which then grows into a long tube as a result of further polymerization.

Like microfilaments, microtubules are dynamic structures with (+) and (–) ends. The (–) end is usually stabilized by bonding to the centrosome. The (+) end shows *dynamic instability*. It can either grow slowly or shorten rapidly. GTP, which is bound by the microtubules and gradually hydrolyzed into GDP, plays a role in this. Various proteins can also be associated with microtubules.



Structure and functions

The cytoskeleton carries out three major tasks:

- It represents the cell's **mechanical scaffolding**, which gives it its typical shape and connects membranes and organelles to each other. This scaffolding has dynamic properties; it is constantly being synthesized and broken down to meet the cell's requirements and changing conditions.
- It acts as the **motor for movement** of animal cells. Not only muscle cells (see p. 332), but also cells of noncontractile tissues contain many different *motor proteins,* which they use to achieve coordinated and directed movement. Cell movement, shape changes during growth, cytoplasmic streaming, and cell division are all made possible by components of the cytoskeleton.
- It serves as a **transport track** within the cell. Organelles and other large protein complexes can move along the filaments with the help of the motor proteins.

A. Microfilaments and intermediate filaments ○

The illustration schematically shows a detail of the **microvilli** of an intestinal epithelial cell as an example of the structure and function of the components of the cytoskeleton (see also **C1**).

Microfilaments of *F actin* traverse the microvilli in ordered bundles. The microfilaments are attached to each other by actin–associated proteins, particularly *fimbrin* and *villin. Calmodulin* and a myosin–like *ATPase* connect the microfilaments laterally to the plasma membrane. *Fodrin,* another microfilament–associated protein, anchors the actin fibers to each other at the base, as well as attaching them to the cytoplasmic membrane and to a network of **intermediate filaments.** In this example, the microfilaments have a mainly static function. In other cases, actin is also involved in dynamic processes. These include muscle contraction (see p. 332), cell movement, phagocytosis by immune cells, the formation of microspikes and lamellipodia (cellular extensions), and the acrosomal process during the fusion of sperm with the egg cell.

B. Microtubules ○

Only the cell's **microtubules** are shown here. They radiate out in all directions from a center near the nucleus, the **centrosome**. The tube-shaped microtubules are constantly being synthesized and broken down at their (+) ends. In the centriole, the (–) end is blocked by associated proteins (see p. 204). The (+) end can also be stabilized by associated proteins—e.g., when the microtubules have reached the cytoplasmic membrane.

The microtubules are involved in defining the shape of the cell and also serve as guiding tracks for the transport of organelles. Together with associated proteins (*dynein, kinesin*), microtubules are able to carry out mechanical work—e.g., during the transport of mitochondria, the movement of cilia (hair-like cell protrusions in the lungs, intestinal epithelium, and oviduct) and the beating of the flagella of sperm. Microtubules also play a special role in the mitotic period of cell division (see p. 394).

C. Architecture ○

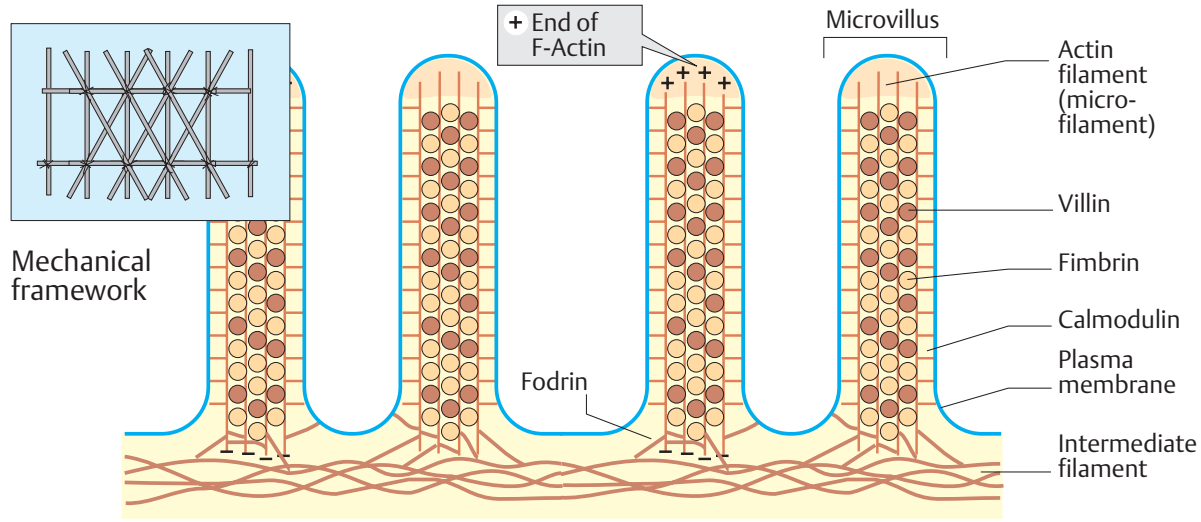
The complex structure and net-like density of the cytoskeleton is illustrated here using three examples in which the cytoskeletal components are visualized with the help of antibodies.

1. The border of an *intestinal epithelial cell* is seen here (see also **B**). There are **microfilaments** (**a**) passing from the interior of the cell out into the microvilli. The filaments are firmly held together by **spectrin** (**b**), an associated protein, and they are anchored to **intermediate filaments** (**c**).

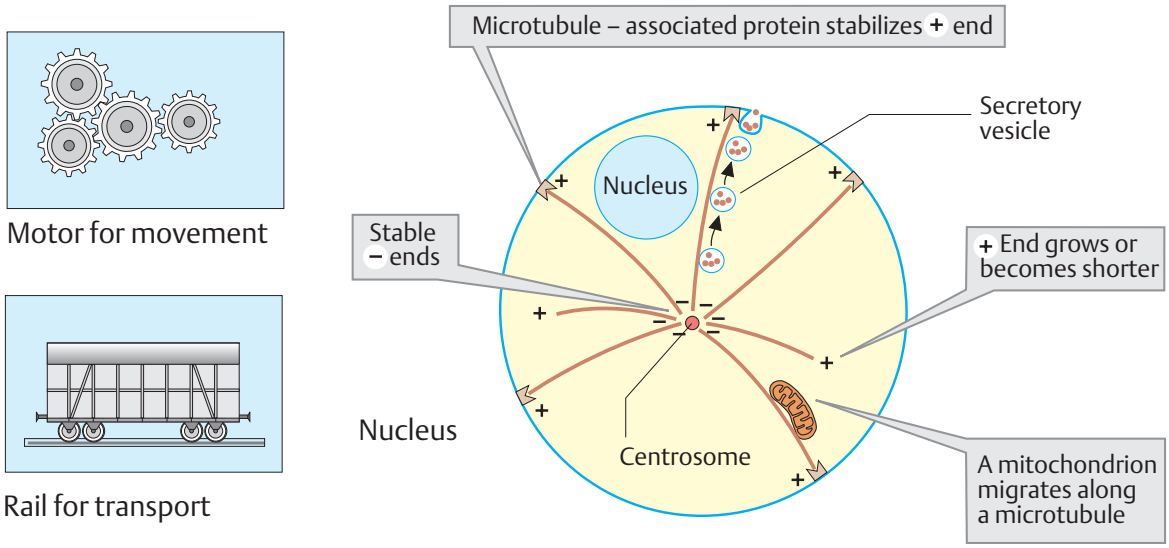
2. Only **microtubules** are seen in this *fibroblast cell.* They originate from the microtubule organizing center (centrosome) and radiate out as far as the plasma membrane.

3. **Keratin filaments** are visible here in an *epithelial cell.* Keratin fibers belong to the group of **intermediate filaments** (see pp. 70, 204; **d** = nucleus).

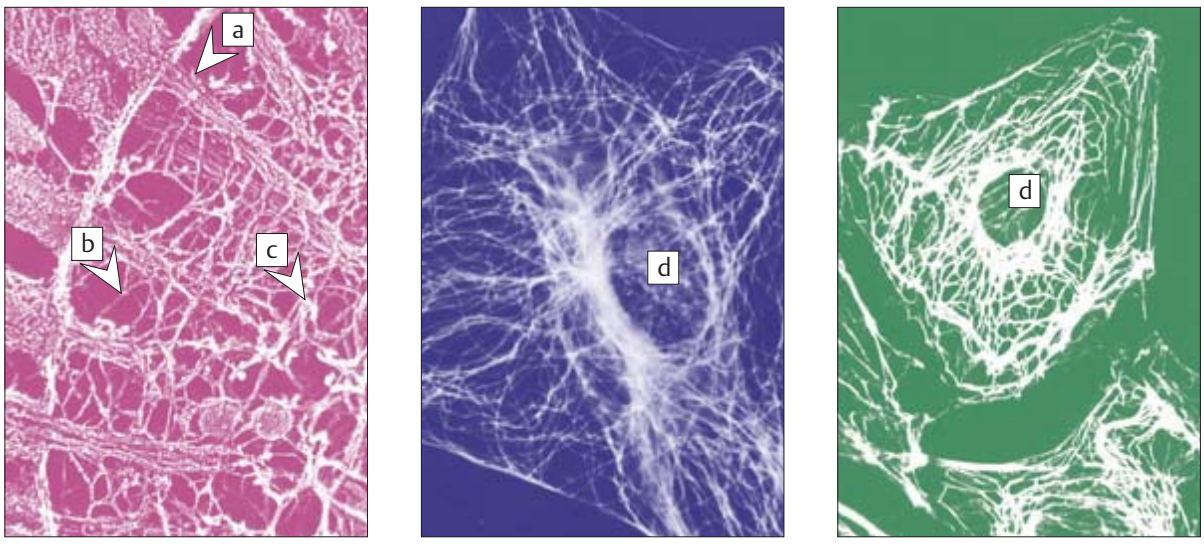
A. Microfilaments and intermediate filaments



B. Microtubules



C. Architecture



1. Microfilaments **2.** Microtubules **3.** Intermediate filaments

Nucleus

A. Nucleus ●

The nucleus is the largest organelle in the eukaryotic cell. With a diameter of about 10 μm, it is easily recognizable with the light microscope. This is the location for *storage, replication,* and *expression* of genetic information.

The nucleus is separated from the cytoplasm by the **nuclear envelope**, which consists of the **outer** and **inner nuclear membranes**. Each of the two nuclear membranes has two layers, and the membranes are separated from each other by the **perinuclear space**. The outer nuclear membrane is continuous with the rough endoplasmic reticulum and is covered with ribosomes. The inner side of the membrane is covered with a protein layer (the nuclear lamina), in which the nuclear structures are anchored.

The nucleus contains almost all of the cell's **DNA** (around 1% of which is mitochondrial DNA). Together with histones and structural proteins, the nuclear DNA forms the **chromatin** (see p. 238). It is only during cell division that chromatin condenses into *chromosomes,* which are also visible with the light microscope. During this phase, the nuclear membrane temporarily disintegrates.

During the phase between cell divisions, the *interphase,* it is possible to distinguish between the more densely packed **heterochromatin** and loose **euchromatin** using an electron microscope. Active *transcription* of DNA into mRNA takes place in the region of the euchromatin. A particularly electron-dense region is noticeable in many nuclei—the **nucleolus** (several nucleoli are sometimes present). The DNA in the nucleolus contains numerous copies of the genes for rRNAs (see p. 242). They are constantly undergoing transcription, leading to a high local concentration of RNA.

B. Nuclear pores ◑

The exchange of substances between the nucleus and the cytoplasm is mediated by **pore complexes** with complicated structures, which traverse the nuclear membrane. The nuclear pores consist of numerous proteins that form several connected rings of varying diameter. Low-molecular structures and small proteins can enter the nucleus without difficulty. By contrast, larger proteins (over 40 kDa) can only pass through the nuclear pores if they carry a **nuclear localization sequence** consisting of four successive basic amino acids inside their peptide chains (see p. 228). mRNAs and rRNAs formed in the nucleus cross the pores into the cytoplasm as complexes with proteins (see below).

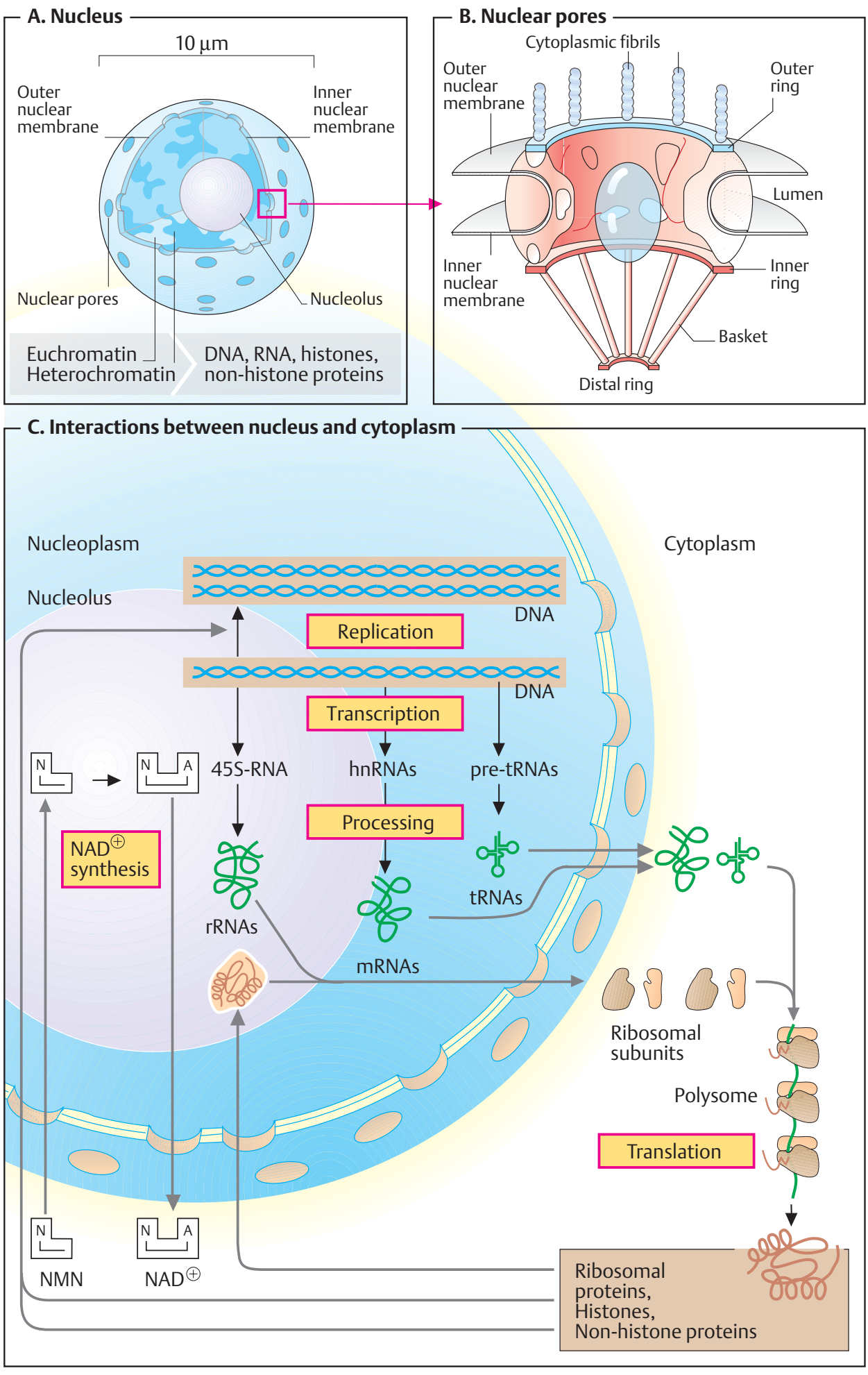
C. Relationships between the nucleus and cytoplasm ◑

Almost all of the RNA in the cell is synthesized in the nucleus. In this process, known as **transcription**, the information stored in DNA is transcribed into RNA (see p. 242). As mentioned above, *ribosomal RNA* (rRNA) is mainly produced in the nucleolus, while *messenger* and *transfer RNA* (mRNA and tRNA) are formed in the region of the euchromatin. Enzymatic duplication of DNA—**replication**—also only takes place in the nucleus (see p. 240).

The nucleotide components required for transcription and replication have to be imported into the nucleus from the cytoplasm. Incorporation of these components into RNA leads to primary products, which are then altered by cleavage, excision of introns, and the addition of extra nucleotides (**RNA maturation**; see p. 242). It is only once these process have been completed that the RNA molecules formed in the nucleus can be exported into the cytoplasm for protein synthesis (**translation**; see p. 250).

The nucleus is not capable of synthesizing proteins. All of the nuclear proteins therefore have to be imported—the *histones* with which DNA is associated in chromatin, and also the so–called *non–histone proteins* (DNA polymerases and RNA polymerases, auxiliary and structural proteins, transcription factors, and ribosomal proteins). Ribosomal RNA (rRNA) already associates with proteins in the nucleolus to form ribosome precursors.

A special metabolic task carried out by the nucleus is **biosynthesis of NAD^+**. The immediate precursor of this coenzyme, *nicotinamide mononucleotide* (NMN^+), arises in the cytoplasm and is then transported into the nucleolus, where it is enzymatically converted into the dinucleotide NAD^+. Finally, NAD^+ then returns to the cytoplasm.



Structure and functions

A. Mitochondrial structure ◑

Mitochondria are bacteria-sized organelles (about 1 × 2 μm in size), which are found in large numbers in almost all eukaryotic cells. Typically, there are about 2000 mitochondria per cell, representing around 25% of the cell volume. Mitochondria are enclosed by two membranes—a smooth **outer** membrane and a markedly folded or tubular **inner mitochondrial membrane**, which has a large surface and encloses the **matrix space**. The folds of the inner membrane are known as **cristae**, and tube-like protrusions are called **tubules**. The **intermembrane space** is located between the inner and the outer membranes.

The number and shape of the mitochondria, as well as the numbers of cristae they have, can differ widely from cell type to cell type. Tissues with intensive oxidative metabolism—e.g., heart muscle—have mitochondria with particularly large numbers of cristae. Even within one type of tissue, the shape of the mitochondria can vary depending on their functional status. Mitochondria are mobile, plastic organelles.

Mitochondria probably developed during an early phase of evolution from aerobic bacteria that entered into symbiosis with primeval anaerobic eukaryotes. This **endosymbiont theory** is supported by many findings. For example, mitochondria have a ring-shaped DNA (four molecules per mitochondrion) and have their own ribosomes. The mitochondrial genome became smaller and smaller during the course of evolution. In humans, it still contains 16 569 base pairs, which code for two rRNAs, 22 tRNAs, and 13 proteins. Only these 13 proteins (mostly subunits of respiratory chain complexes) are produced in the mitochondrion. All of the other mitochondrial proteins are coded by the nuclear genome and have to be imported into the mitochondria after translation in the cytoplasm (see p. 228). The mitochondrial envelope consisting of two membranes also supports the endosymbiont theory. The inner membrane, derived from the former symbiont, has a structure reminiscent of prokaryotes. It contains the unusual lipid cardiolipin (see p. 50), but hardly any cholesterol (see p. 216).

Both mitochondrial membranes are very rich in proteins. **Porins** (see p. 214) in the outer membrane allow small molecules (< 10 kDa) to be exchanged between the cytoplasm and the intermembrane space. By contrast, the inner mitochondrial membrane is completely impermeable even to small molecules (with the exception of O_2, CO_2, and H_2O). Numerous **transporters** in the inner membrane ensure the import and export of important metabolites (see p. 212). The inner membrane also transports **respiratory chain** complexes, **ATP synthase**, and other enzymes. The matrix is also rich in enzymes (see **B**).

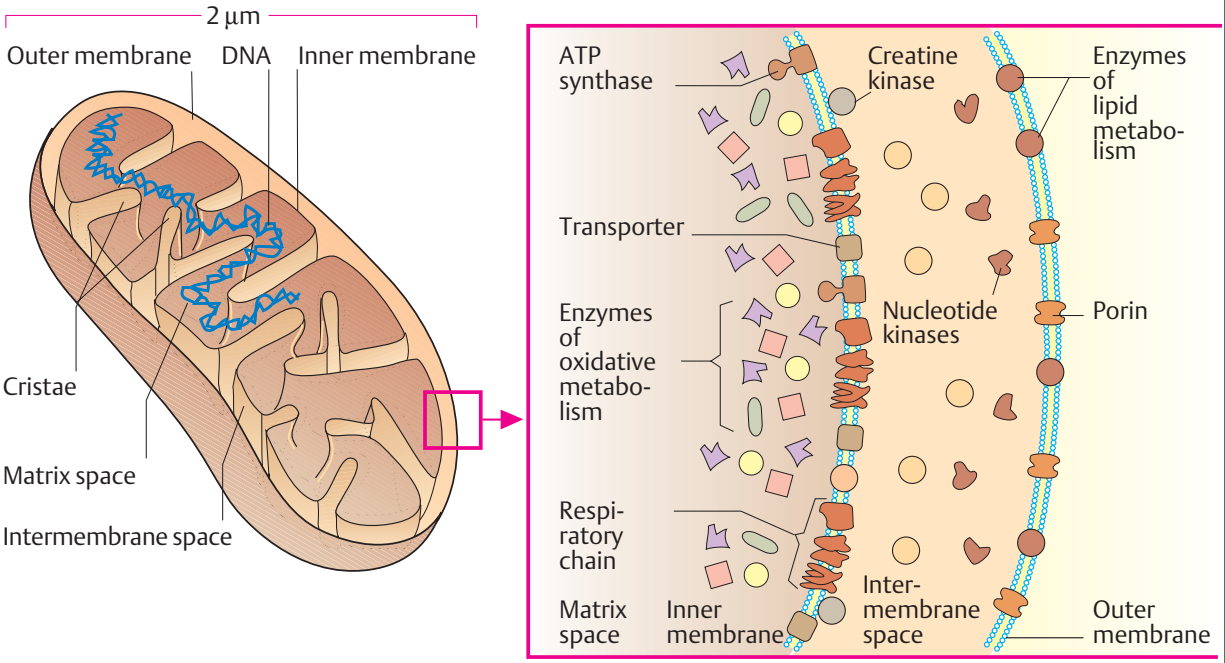
B. Metabolic functions ◑

Mitochondria are also described as being the cell's *biochemical powerhouse,* since—through **oxidative phosphorylation** (see p. 112)—they produce the majority of cellular ATP. **Pyruvate dehydrogenase** (PDH), the **tricarboxylic acid cycle**, β-**oxidation** of fatty acids, and parts of the **urea cycle** are located in the matrix. The **respiratory chain**, **ATP synthesis**, and enzymes involved in **heme biosynthesis** (see p. 192) are associated with the inner membrane.

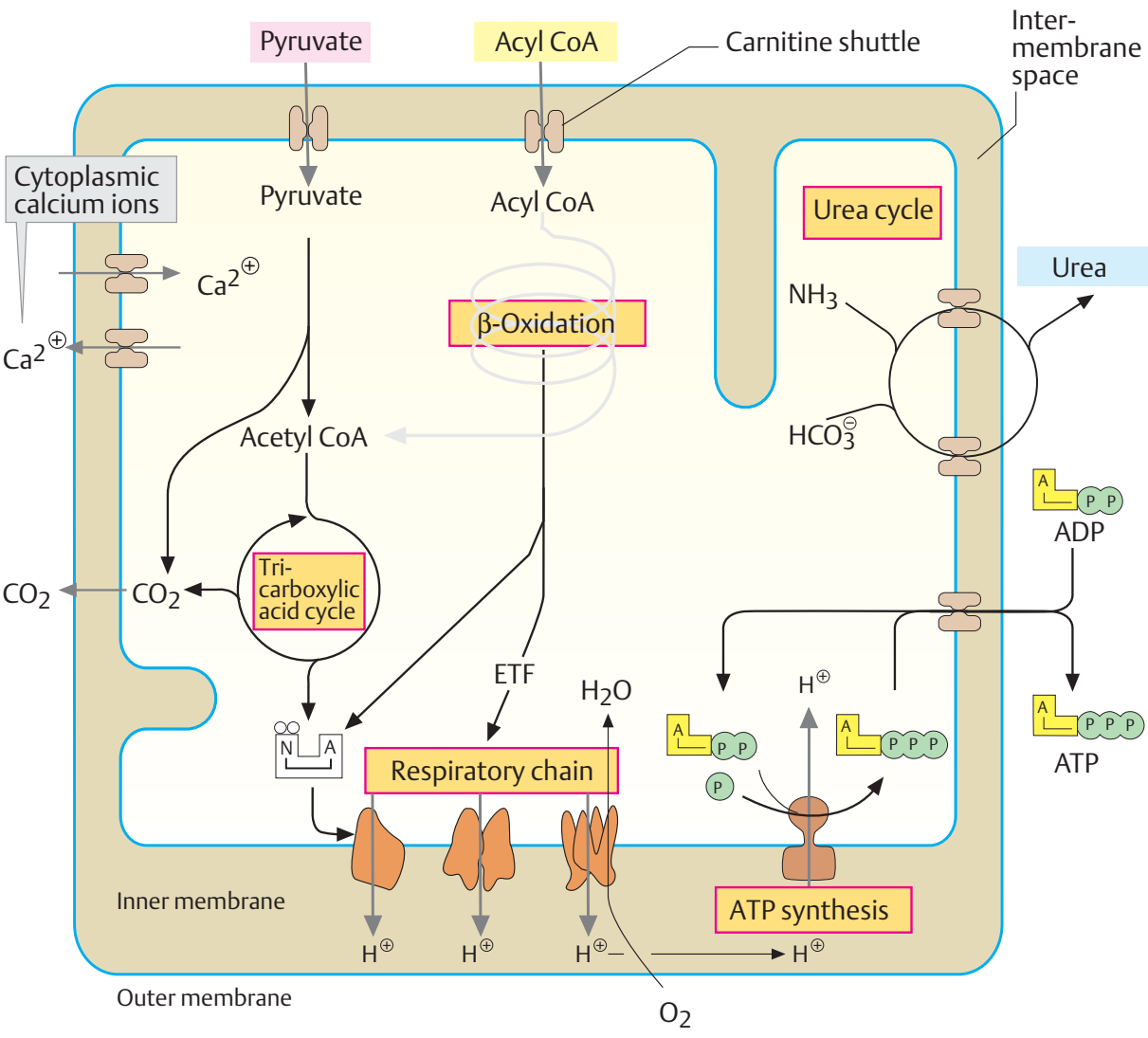
The inner membrane itself plays an important part in oxidative phosphorylation. As it is impermeable to protons, the respiratory chain—which pumps protons from the matrix into the intermembrane space via complexes I, III, and IV—establishes a **proton gradient** across the inner membrane, in which the chemical energy released during NADH oxidation is conserved (see p. 126). ATP synthase then uses the energy stored in the gradient to form ATP from ADP and inorganic phosphate. Several of the transport systems are also dependent on the H^+ gradient.

In addition to the endoplasmic reticulum, the mitochondria also function as an intracellular **calcium reservoir**. The mitochondria also play an important role in "programmed cell death"—**apoptosis** (see p. 396).

A. Mitochondrial structure



B. Metabolic functions



Transport systems

Mitochondria are surrounded by an inner and an outer membrane (see p. 210). The **outer membrane** contains porins, which allow smaller molecules up to 10 kDa in size to pass. By contrast, the **inner membrane** is also impermeable to small molecules (with the exception of water and the gases O_2, CO_2, and NH_3). All of the other substrates of mitochondrial metabolism, as well as its products, therefore have to be moved through the inner membrane with the help of special **transporters**.

A. Transport systems ◑

The transport systems of the inner mitochondrial membrane use various mechanisms. Metabolites or ions can be transported alone (uniport, **U**), together with a second substance (symport, **S**), or in exchange for another molecule (antiport, **A**). Active transport—i. e., transport coupled to ATP hydrolysis—does not play an important role in mitochondria. The driving force is usually the **proton gradient** across the inner membrane (blue star) or the general membrane potential (red star; see p. 126).

The **pyruvate** (left) formed by glycolysis in the cytoplasm is imported into the matrix in antiport with OH^-. The OH^- ions react in the intermembrane space with the H^+ ions abundantly present there to form H_2O. This maintains a concentration gradient of OH^-. The import of **phosphate** ($H_2PO_4^-$) is driven in a similar way. The exchange of the **ATP** formed in the mitochondrion for **ADP** via an *adenine nucleotide translocase* (center) is also dependent on the H^+ gradient. ATP with a quadruple negative charge is exchanged for ADP with a triple negative charge, so that overall one negative charge is transported into the H^+-rich intermembrane space. The import of malate by the *tricarboxylate transporter*, which is important for the malate shuttle (see **B**) is coupled to the export of citrate, with a net export of one negative charge to the exterior again. In the opposite direction, malate can leave the matrix in antiport for phosphate. When Ca^{2+} is imported (right), the metal cation follows the membrane potential. An electroneutral antiport for two H^+ or two Na^+ serves for Ca^{2+} export.

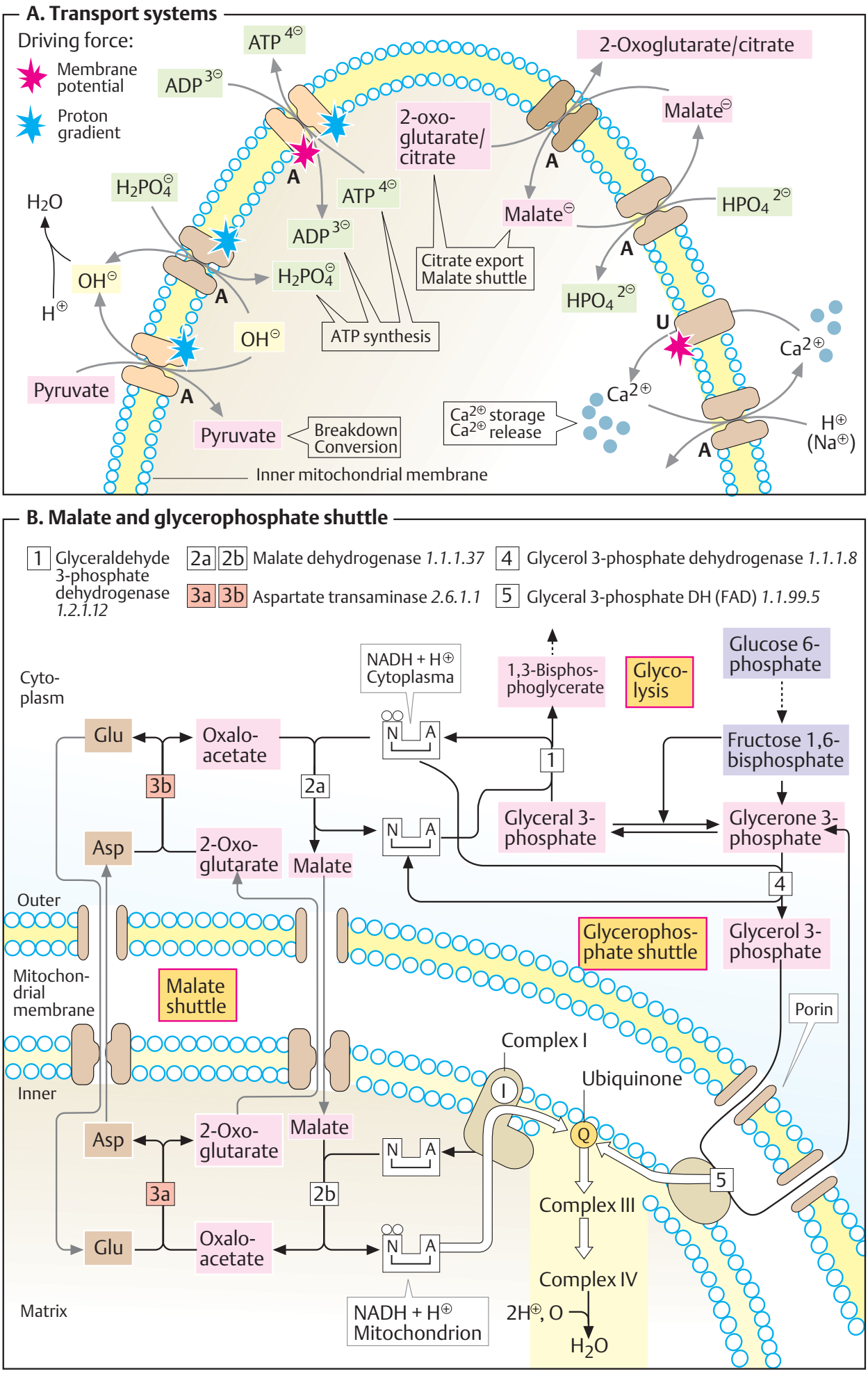
B. Malate and glycerophosphate shuttles ◑

Two systems known as "shuttles" are available to allow the **import of reducing equivalents** that arise from glycolysis in the cytoplasm in the form of $NADH+H^+$. There is no transporter in the inner membrane for $NADH+H^+$ itself.

In the **malate shuttle** (left)—which operates in the heart, liver, and kidneys, for example—oxaloacetic acid is reduced to malate by malate dehydrogenase (MDH, [2a]) with the help of $NADH+H^+$. In antiport for 2-oxoglutarate, malate is transferred to the matrix, where the mitochondrial isoenzyme for MDH [2b] regenerates oxaloacetic acid and $NADH+H^+$. The latter is reoxidized by complex I of the respiratory chain, while oxaloacetic acid, for which a transporter is not available in the inner membrane, is first transaminated to aspartate by aspartate aminotransferase (AST, [3a]). Aspartate leaves the matrix again, and in the cytoplasm once again supplies oxaloacetate for step [2a] and glutamate for return transport into the matrix [3b]. On balance, only $NADH+H^+$ is moved from the cytoplasm into the matrix; ATP is not needed for this.

The **glycerophosphate shuttle** (right) was discovered in insect muscle, but is also active in the skeletal musculature and brain in higher animals. In this shuttle, $NADH+H^+$ formed in the cytoplasm is used to reduce glycerone 3-phosphate, an intermediate of glycolysis (see p. 150) to glycerol 3-phosphate. Via porins, this enters the intermembrane space and is oxidized again there on the exterior side of the inner membrane by the flavin enzyme *glycerol 3-phosphate dehydrogenase* back into glycerone 3-phosphate. The reducing equivalents are passed on to the respiratory chain via **ubiquinone (coenzyme Q)**.

The **carnitine shuttle** for transporting acyl residues into the mitochondrial matrix is discussed on p. 164.



Structure and components

A. Structure of the plasma membrane ◑

All biological membranes are constructed according to a standard pattern. They consist of a continuous **bilayer of amphipathic lipids** approximately 5 nm thick, into which **proteins** are embedded. In addition, some membranes also carry **carbohydrates** (mono- and oligosaccharides) on their exterior, which are bound to lipids and proteins. The proportions of lipids, proteins, and carbohydrates differ markedly depending on the type of cell and membrane (see p. 216).

Membrane lipids are strongly *amphipathic molecules* with a polar hydrophilic "head group" and an apolar hydrophobic "tail." In membranes, they are primarily held together by the hydrophobic effect (see p. 28) and weak Van der Waals forces, and are therefore mobile relative to each other. This gives membranes a more or less fluid quality.

The **fluidity** of membranes primarily depends on their lipid composition and on temperature. At a specific **transition temperature**, membranes pass from a semicrystalline state to a more fluid state. The double bonds in the alkyl chains of unsaturated acyl residues in the membrane lipids disturb the semicrystalline state. The *higher* the proportion of unsaturated lipids present, therefore, the *lower* the transition temperature. The cholesterol content also influences membrane fluidity. While cholesterol increases the fluidity of semicrystalline, closely-packed membranes, it *stabilizes* fluid membranes that contain a high proportion of unsaturated lipids.

Like lipids, proteins are also mobile within the membrane. If they are not fixed in place by special mechanisms, they float within the lipid layer as if in a two-dimensional liquid; biological membranes are therefore also described as being a "fluid mosaic."

Lipids and proteins can shift easily *within* one layer of a membrane, but switching between the two layers (*"flip/flop"*) is not possible for proteins and is only possible with difficulty for lipids (with the exception of cholesterol). To move to the other side, phospholipids require special auxiliary proteins (translocators, "flipases").

B. Membrane lipids ◑

The illustration shows a model of a small section of a membrane. The **phospholipids** are the most important group of membrane lipids. They include *phosphatidylcholine* (lecithin), *phosphatidylethanolamine*, *phosphatidylserine, phosphatidylinositol,* and *sphingomyelin* (for their structures, see p. 50). In addition, membranes in animal cells also contain **cholesterol** (with the exception of inner mitochondrial membranes). **Glycolipids** (a *ganglioside* is shown here) are mainly found on the outside of the plasma membrane. Together with the *glycoproteins,* they form the exterior coating of the cell (the *glycocalyx*).

C. Membrane proteins ◑

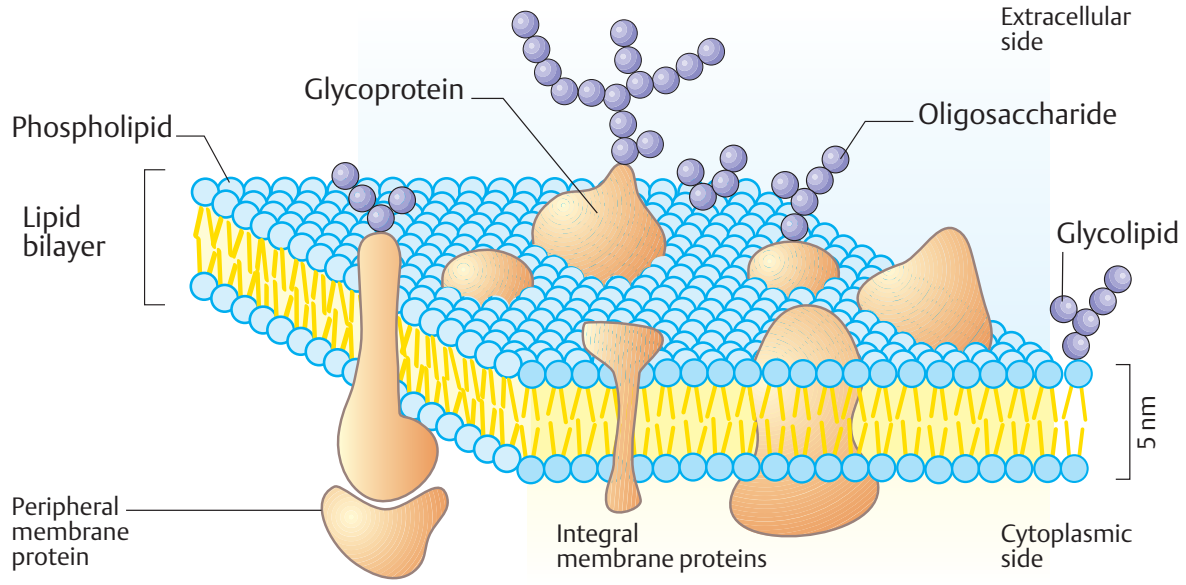
Proteins can be anchored in or on membranes in various ways. **Integral membrane proteins** cross right through the lipid bilayer. The sections of the peptide chains that lie within the bilayer usually consist of 20 to 25 mainly hydrophobic amino acid residues that form a right-handed α-helix.

Type I and II membrane proteins only contain *one* **transmembrane helix** of this type, while type III proteins contain several. Rarely, type I and II polypeptides can aggregate to form a type IV transmembrane protein. Several groups of integral membrane proteins—e.g., the porins (see p. 212)—penetrate the membrane with antiparallel β-sheet structures. Due to its shape, this tertiary structure is known as a "β-barrel."

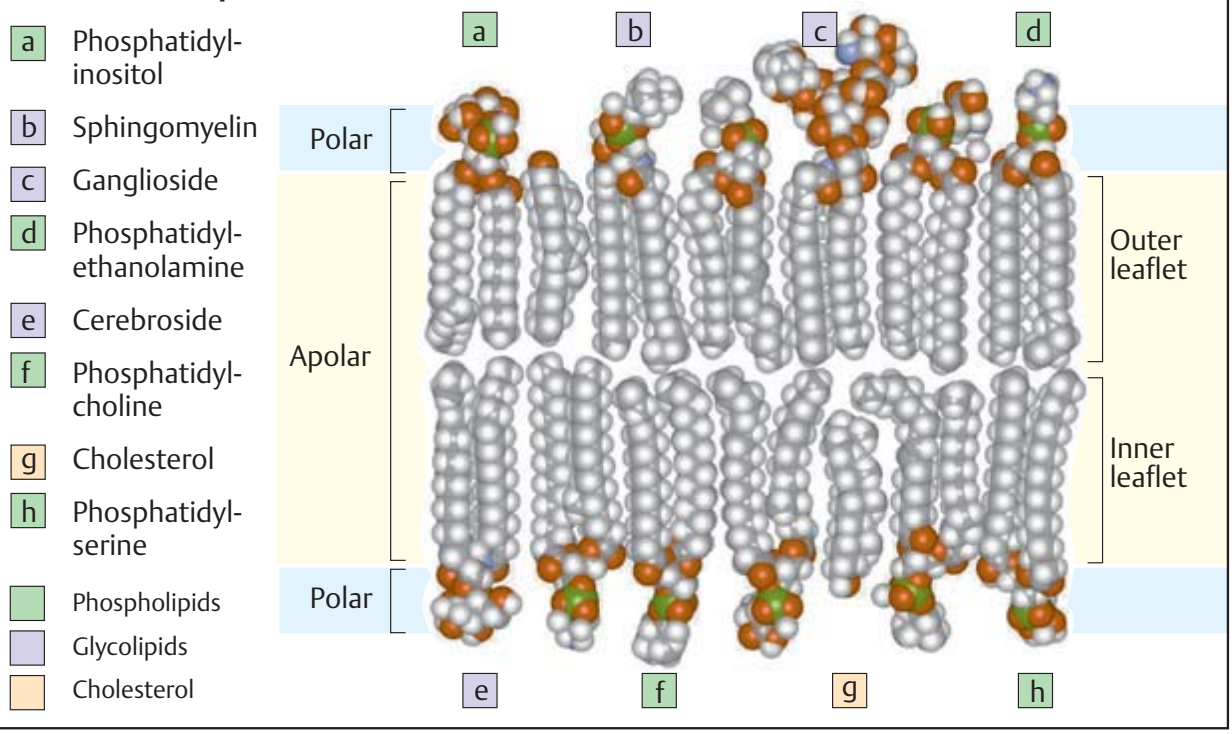
Type V and VI proteins carry **lipid anchors.** These are fatty acids (palmitic acid, myristic acid), isoprenoids (e.g., farnesol), or glycolipids such as glycosyl phosphatidylinositol (GPI) that are covalently bound to the peptide chain.

Peripheral membrane proteins are associated with the head groups of phospholipids or with another integral membrane protein (not shown).

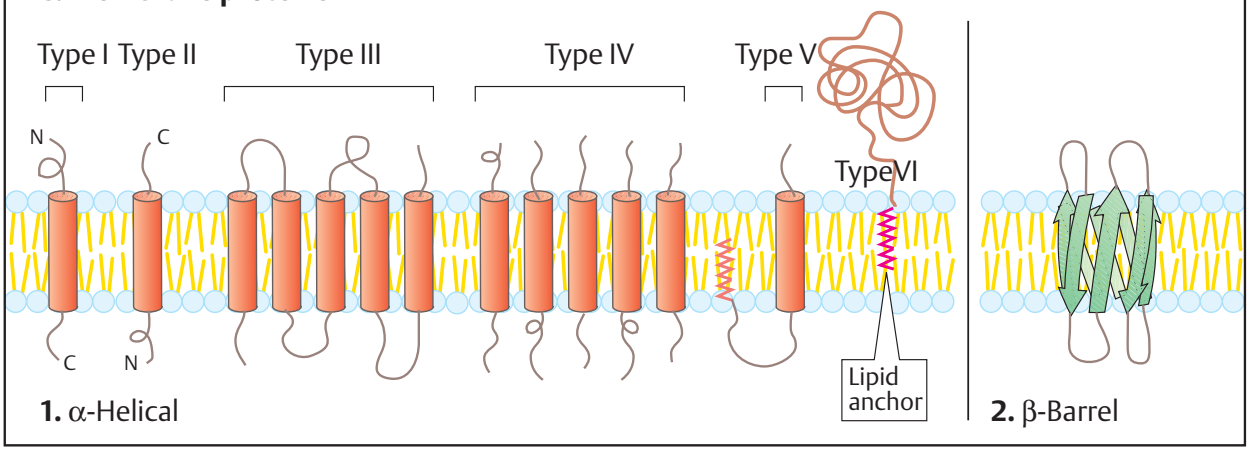
A. Structure of the plasma membrane



B. Membrane lipids



C. Membrane proteins



Functions and composition

The most important membranes in animal cells are the *plasma membrane,* the inner and outer *nuclear membranes,* the membranes of the *endoplasmic reticulum* (ER) and the *Golgi apparatus,* and the inner and outer *mitochondrial membranes*. *Lysosomes, peroxisomes,* and various *vesicles* are also separated from the cytoplasm by membranes. In plants, additional membranes are seen in the plastids and vacuoles. All membranes show *polarity*—i. e., there is a difference in the composition of the inner layer (facing toward the cytoplasm) and the outer layer (facing away from it).

A. Functions of membranes ●

Membranes and their components have the following functions:

1. **Enclosure and insulation** of cells and organelles. The *enclosure* provided by the plasma membrane protects cells from their environment both mechanically and chemically. The plasma membrane is essential for maintaining differences in the concentration of many substances between the intracellular and extracellular compartments.

2. **Regulated transport of substances**, which determines the *internal milieu* and is a precondition for *homeostasis*—i. e., the maintenance of constant concentrations of substances and physiological parameters. Regulated and selective transport of substances through pores, channels, and transporters (see p. 218) is necessary because the cells and organelles are enclosed by membrane systems.

3. **Reception of extracellular signals** and transfer of these signals to the inside of the cell (see pp. 384 ff.), as well as the production of signals.

4. **Enzymatic catalysis** of reactions. Important enzymes are located in membranes at the interface between the lipid and aqueous phases. This is where reactions with apolar substrates occur. Examples include *lipid biosynthesis* (see p. 170) and the *metabolism of apolar xenobiotics* (see p. 316). The most important reactions in energy conversion—i. e., *oxidative phosphorylation* (see p. 140) and *photosynthesis* (see p. 128)—also occur in membranes.

5. **Interactions with other cells** for the purposes of cell fusion and tissue formation, as well as communication with the extracellular matrix.

6. **Anchoring of the cytoskeleton** (see p. 204) to maintain the shape of cells and organelles and to provide the basis for movement processes.

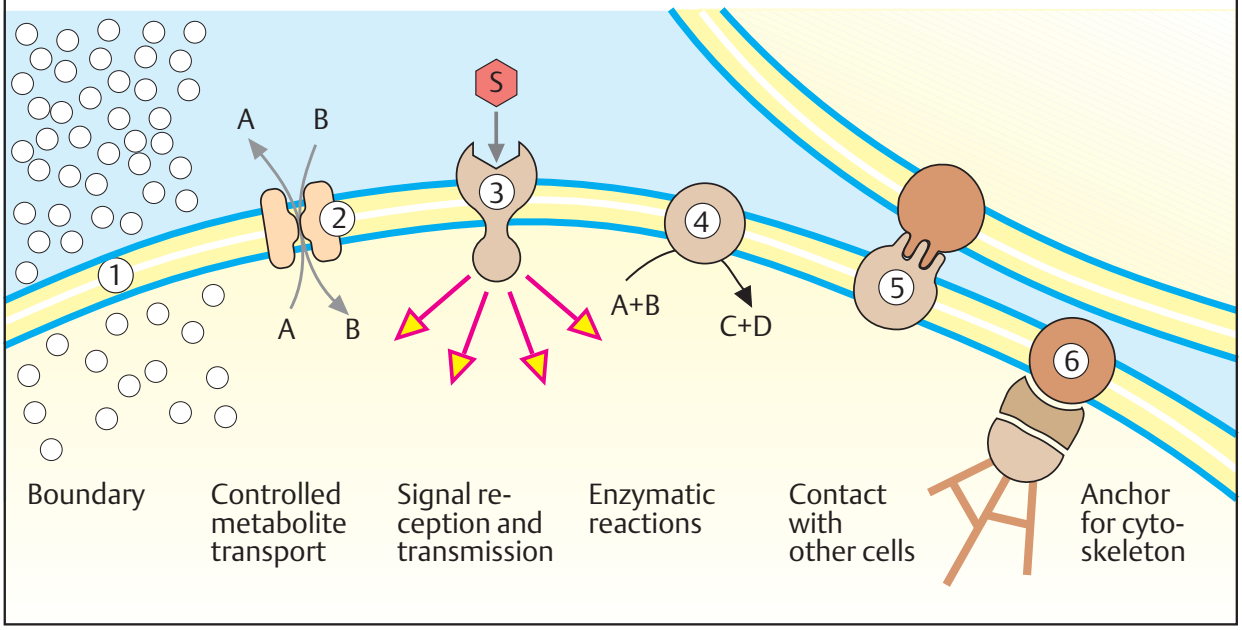
B. Composition of membranes ◑

Biological membranes consist of **lipids**, **proteins**, and **carbohydrates** (see p. 214). These components occur in varying proportions (left). Proteins usually account for the largest proportion, at around half. By contrast, carbohydrates, which are only found on the side facing away from the cytoplasm, make up only a few percent. An extreme composition is seen in *myelin,* the insulating material in nerve cells, three-quarters of which consists of lipids. By contrast, the inner *mitochondrial membrane* is characterized by a very low proportion of lipids and a particularly high proportion of proteins.

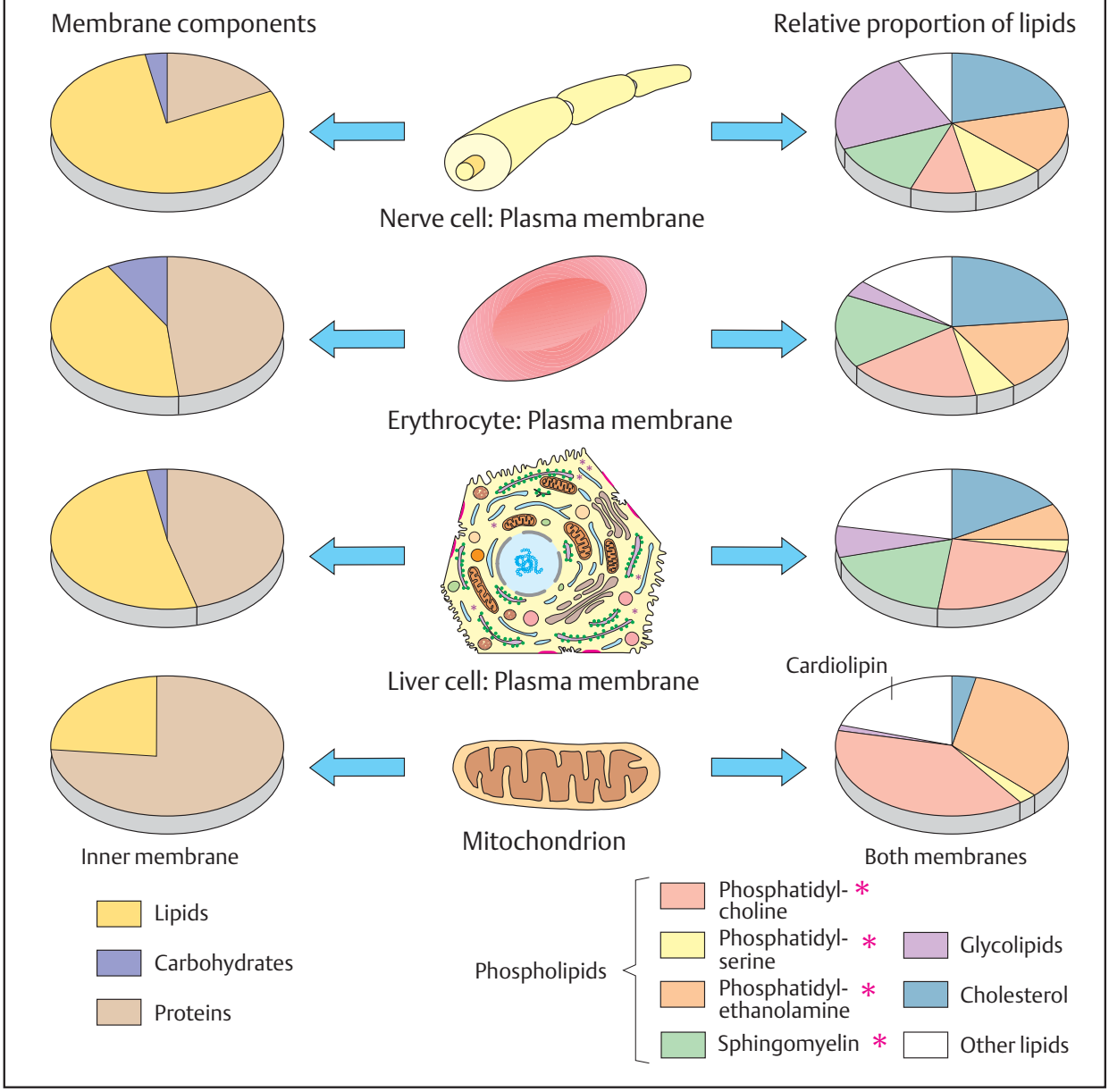
When the individual proportions of **lipids** in membranes are examined more closely (right part of the illustration), typical patterns for particular cells and tissues are also found. The illustration shows the diversity of the membrane lipids and their approximate quantitative composition. *Phospholipids* are predominant in membrane lipids in comparison with *glycolipids* and *cholesterol*. Triacylglycerols (neutral fats) are not found in membranes.

Cholesterol is found almost exclusively in eukaryotic cells. Animal membranes contain substantially more cholesterol than plant membranes, in which cholesterol is usually replaced by other sterols. There is no cholesterol at all in prokaryotes (with a few exceptions). The inner mitochondrial membrane of eukaryotes is also low in cholesterol, while it is the only membrane that contains large amounts of cardiolipin. These facts both support the endosymbiotic theory of the development of mitochondria (see p. 210).

A. Functions of membranes



B. Composition of membranes



Transport processes

A. Permeability ●

Only small, uncharged molecules such as gases, water, ammonia, glycerol, or urea are able to pass through biological membranes by *free diffusion*. With increasing **size**, even compounds of this type are no longer able to pass through. Membranes are impermeable to glucose and other sugars, for example.

The **polarity** of a molecule is also important. Apolar substances, such as benzene, ethanol, diethyl ether, and many narcotic agents are able to enter biological membranes easily. By contrast, membranes are impermeable to strongly polar compounds, particularly those that are electrically charged. To be able to take up or release molecules of this type, cells have specialized *channels* and *transporters* in their membranes (see below).

B. Passive and active transport ●

Free diffusion is the simplest form of membrane transport. When it is supported by integral membrane proteins, it is known as **facilitated diffusion** (or facilitated transport).

1. **Channel proteins** have a polar pore through which ions and other hydrophilic compounds can pass. For example, there are channels that allow selected ions to pass (**ion channels**; see p. 222) and **porins** that allow molecules below a specific size to pass in a more or less nonspecific fashion (see p. 212).
2. **Transporters** recognize and bind the molecules to be transported and help them to pass through the membrane as a result of a conformational change. These proteins (permeases) are thus comparable with enzymes—although with the difference that they "catalyze" vectorial transport rather than an enzymatic reaction. Like enzymes, they show a certain *affinity* for each molecule transported (expressed as the dissociation constant, K_d in mol L^{-1}) and a *maximum transport capacity* (V).

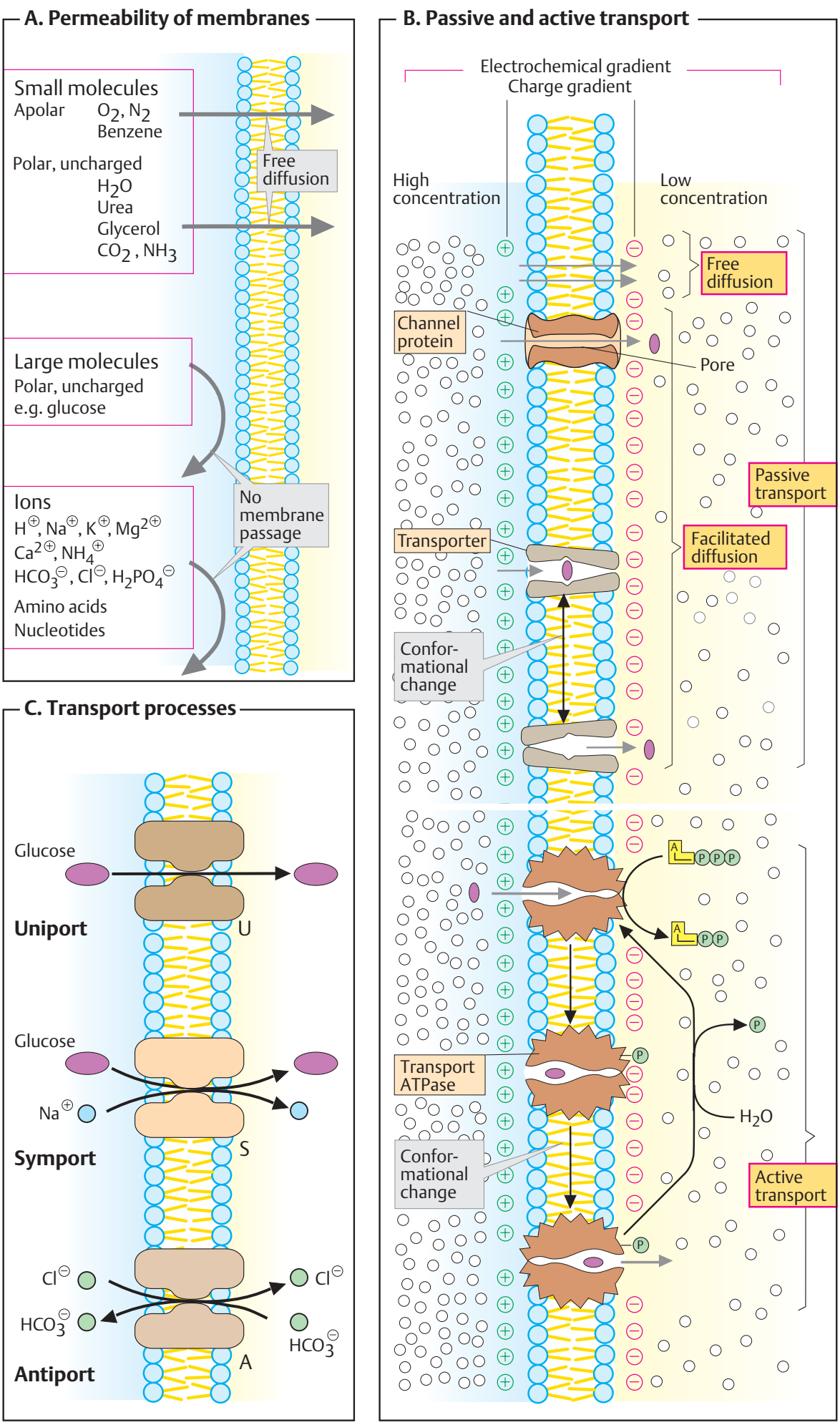
Free diffusion and transport processes facilitated by ion channels and transport proteins always follow a *concentration gradient*—i.e., the direction of transport is from the site of higher concentration to the site of lower concentration. In ions, the *membrane potential* also plays a role; the processes are summed up by the term *"electrochemical gradient"* (see p. 126). These processes therefore involve **passive transport**, which runs "downhill" on the slope of a gradient.

By contrast, **active transport** can also run "uphill"—i.e., against a concentration or charge gradient. It therefore requires an input of *energy,* which is usually supplied by the hydrolysis of ATP (see p. 124). The transporter first binds its "cargo" on one side of the membrane. ATP-dependent phosphorylation then causes a conformation change that releases the cargo on the other side of the membrane (see p. 220). A non-spontaneous transport process can also take place through coupling to another active transport process (known as *secondary active transport;* see p. 220).

Using the transport systems in the membranes, cells regulate their volume, internal pH value, and ionic environment. They concentrate metabolites that are important for energy metabolism and biosynthesis, and exclude toxic substances. Transport systems also serve to establish *ion gradients,* which are required for oxidative phosphorylation and stimulation of muscle and nerve cells, for example (see p. 350).

C. Transport processes ◑

Another classification of transport processes is based on the number of particles transported and the direction in which they move. When a *single* molecule or ion passes through the membrane with the help of a channel or transporter, the process is described as a **uniport** (example: the transport of glucose into liver cells). Simultaneous transport of *two different* particles can take place either as a **symport** (example: the transport of amino acids or glucose together with Na^+ ions into intestinal epithelial cells) or as an **antiport**. Ions are often transported in an antiport in exchange for another similarly charged ion. This process is **electroneutral** and therefore more energetically favorable (example: the exchange of HCO_3^- for Cl^- at the erythrocyte membrane).



Transport proteins

Illustrations **B–D** show transporters whose structure has been determined experimentally or established on analogy with other known structures. They all belong to group III of the α-helical transmembrane proteins (see p. 214).

A. Transport mechanisms ◑

Some cells couple the "pure" transport forms discussed on p. 218—i. e., passive transport (**1**) and active transport (**2**)—and use this mechanism to take up metabolites. In **secondary active transport** (**3**), which is used for example by epithelial cells in the small intestine and kidney to take up glucose and amino acids, there is a **symport** (S) located on the luminal side of the membrane, which takes up the metabolite M together with an Na^+ ion. An ATP-dependent Na^+ transporter (Na^+/K^+ ATPase; see p. 350) on the other side keeps the intracellular Na+ concentration low and thus indirectly drives the uptake of M. Finally, a uniport (U) releases M into the blood.

B. Glucose transporter Glut-1 ○

The glucose transporters (Glut) are a family of related membrane proteins with varying distribution in the organs. Glut-1 and Glut-3 have a relatively high af nity for glucose (K_d = 1 mM). They occur in nearly all cells, and ensure continuous glucose uptake. Glut-2 is found in the liver and pancreas. This form has a lower af nity (K_d = 15–20 mM). The rate of glucose uptake by Glut-2 is therefore strongly dependent on the blood glucose level (normally 4–8 mM). Transport by Glut-4 (K_d = 5 mM), which is mainly expressed in muscle and fat cells, is controlled by insulin, which increases the number of transporters on the cell surface (see p. 388). Glut-5 mediates secondary active resorption of glucose in the intestines and kidney (see **A**).

Glut-1 consists of a single peptide chain that spans the membrane with 12 α-helices of different lengths. The glucose is bound by the peptide loops that project on each side of the membrane.

C. Aquaporin-1 ○

Aquaporins help water to pass through biological membranes. They form hydrophilic pores that allow H_2O molecules, but not hydrated ions or larger molecules, to pass through. Aquaporins are particularly important in the kidney, where they promote the reuptake of water (see p. 328). Aquaporin-2 in the renal collecting ducts is regulated by **antidiuretic hormone** (ADH, vasopressin), which via cAMP leads to shifting of the channels from the ER into the plasma membrane.

Aquaporin-1, shown here, occurs in the proximal tubule and in Henle's loop. It contains eight transmembrane helices with different lengths and orientations. The yellow-colored residues form a narrowing that only H_2O molecules can overcome.

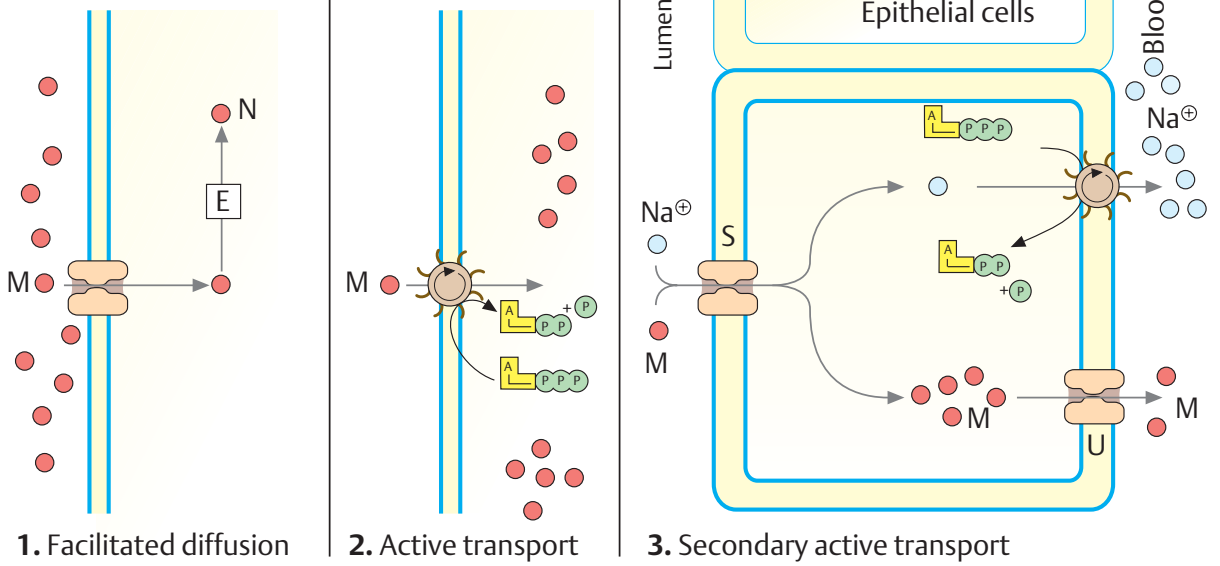
D. Sarcoplasmic Ca^{2+} pump ○

Transport ATPases transport cations—they are "ion pumps." ATPases of the **F type**—e. g., mitochondrial ATP synthase (see p. 142)—use H^+ transport for *ATP synthesis.* Enzymes of the **V type**, using up ATP, "pump" protons into lysosomes and other acidic cell compartments (see p. 234). **P type** transport ATPases are particularly numerous. These are ATP-driven cation transporters that undergo covalent phosphorylation during the transport cycle.

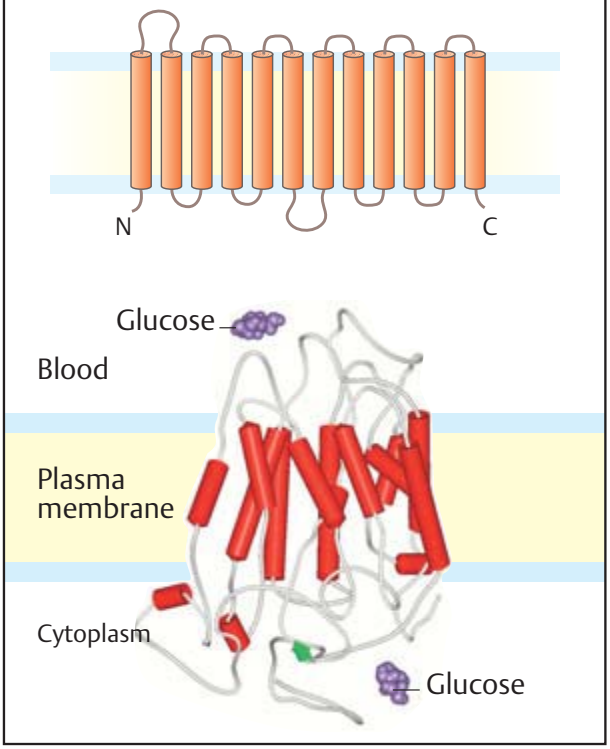
The **Ca^{2+} ATPase** shown also belongs to the P type. In muscle, its task is to pump the Ca^{2+} released into the cytoplasm to trigger muscle contraction back into the sarcoplasmic reticulum (SR; see p. 334). The molecule (**1**) consists of a single peptide chain that is folded into various domains. In the transmembrane part, which is formed by numerous α-helices, there are binding sites for two Ca^{2+} ions (blue) ATP is bound to the cytoplasmic N domain (green).

Four different stages can be distinguished in the enzyme's catalytic cycle (**2**). First, binding of ATP to the N domain leads to the uptake of two Ca^{2+} into the transmembrane part (**a**). Phosphorylation of an aspartate residue in the P domain (**b**) and dissociation of ADP then causes a conformation change that releases the Ca^{2+} ions into the SR (**c**). Finally, dephosphorylation of the aspartate residue restores the initial conditions (**d**).

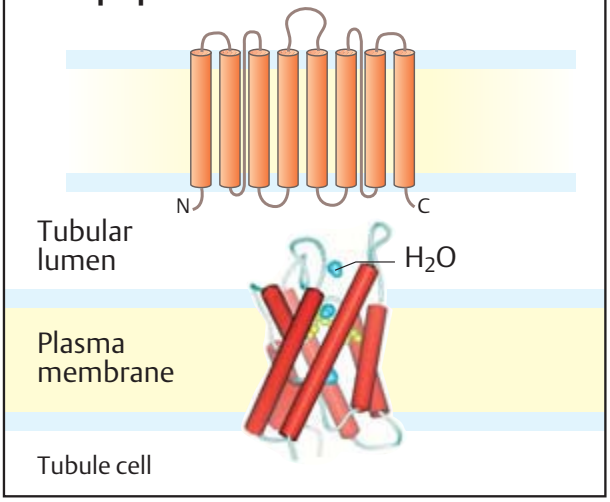
A. Transport mechanisms



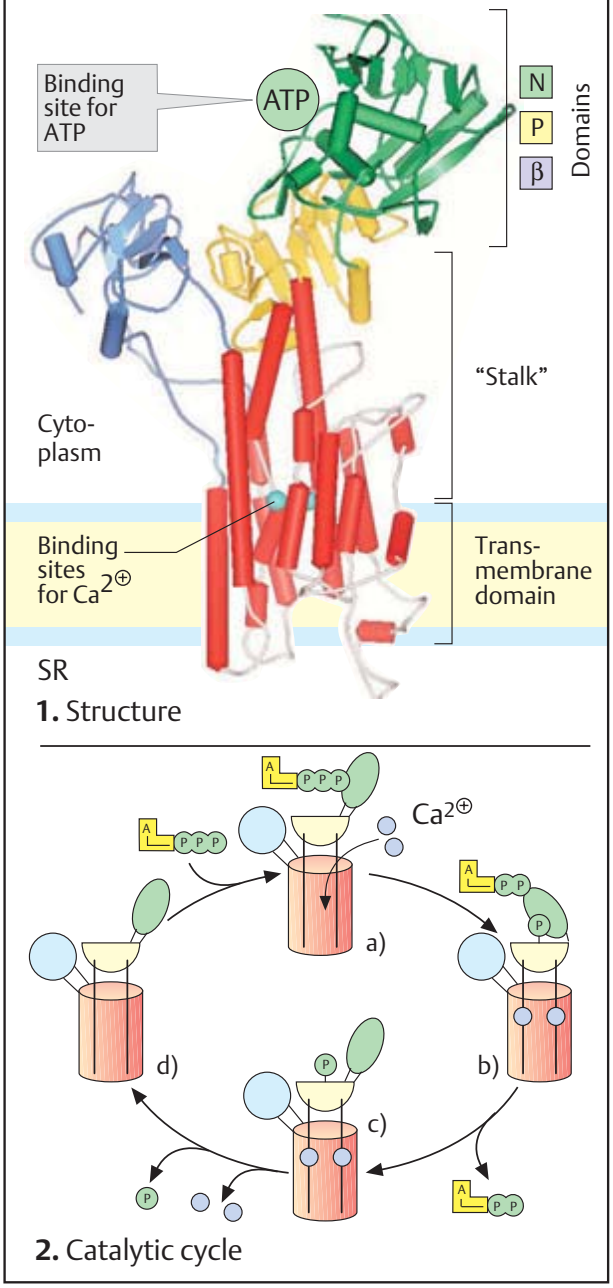
B. Glucose transporter Glut-1



C. Aquaporin-1



D. Sarcoplasmic $Ca^{2\oplus}$ pump



Ion channels

Ion channels facilitate the diffusion of ions through biological membranes. Some ion channels open and close depending on the membrane potential (**voltage-gated channels, A**) or in response to specific ligands (**ligand-gated channels, B**). Other channels operate passively. In these cases, transport depends only on the concentration gradient (**C**).

A. Voltage-gated Na^+ channel ○

Voltage-gated Na^+ channels play a decisive part in the conduction of electrical impulses in the nervous system (see p. 350). These channels open when the membrane potential in their environment reverses. Due to the high equilibrium potential for Na^+ (see p. 126), an inflow of Na^+ ions takes place, resulting in local **depolarization** of the membrane, which propagates by activation of neighboring voltage-dependent Na^+ channels. A spreading depolarization wave of this type is known as an **action potential** (see p. 350). Externally directed K^+ channels are involved in the repolarization of the membrane. In their functioning, these resemble the much more simply structured K^+ channels shown in **C**. The Ca^{2+} channels that trigger exocytosis of vesicles (see p. 228) are also controlled by the action potential.

The voltage-gated Na^+ channels in higher animals are large complexes made up of several subunits. The α-subunit shown here mediates Na^+ transport. It consists of a very long peptide chain (around 2000 amino acid residues), which is folded into four domains, each with six transmembrane helices (left). The S6 helices of all the domains (blue) together form a centrally located hydrophilic pore which can be made narrow or wide depending on the channel's functional status. The six S4 helices (green) function as voltage sensors.

The current conception of the way in which the opening and closing mechanism functions is shown in a highly simplified form on the right. For the sake of clarity, only one of the four domains (domain IV) is shown. The S4 helices contain several positively charged residues. When the membrane is polarized (**a**), the surplus negative charges on the inner side keep the helix in the membrane. If this attraction is removed as a result of local depolarization, the S4 helices are thought to snap upwards like springs and thus open the central pore (**b**).

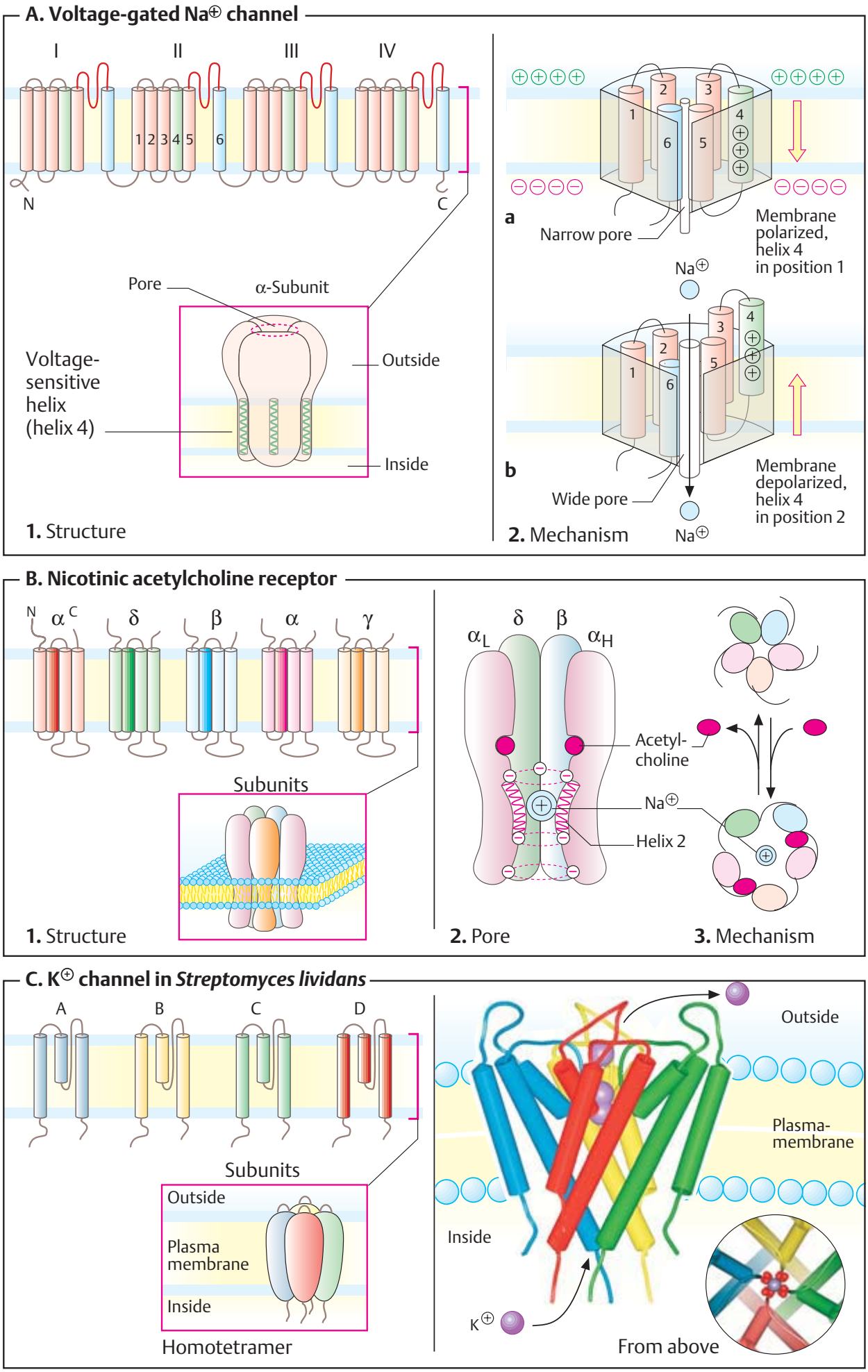
B. Nicotinic acetylcholine receptor ○

Many receptors for neurotransmitters function as ligand-gated channels for Na^+, K^+ and/or Ca^{2+} ions (see p. 354). The ones that have been studied in the greatest detail are the nicotinic receptors for **acetylcholine** (see p. 352). These consist of five separate but structurally closely related subunits. Each forms four transmembrane helices, the second of which is involved in the central pore in each case. The type of monomer and its arrangement in the complex is not identical in all receptors of this type. In the neuromuscular junction (see p. 334), the arrangement $\alpha\beta\gamma\alpha\delta$ is found (**1**).

In the interior of the structure, acetylcholine binds to the two α-subunits and thus opens the pore for a short time (1–2 ms). Negatively charged residues are arranged in three groups in a ring shape inside it. They are responsible for the receptor's ion specificity. It is thought that binding of the neurotransmitter changes the position of the subunits in such a way that the pore expands (**3**). The bound acetylcholine dissociates again and is hydrolytically inactivated (see p. 356). The receptor is thus able to function again.

C. K^+ channel in *Streptomyces lividans* ○

The only detailed structures of ion channels established so far are those of potassium channels like that of an outwardly directed K^+ channel in the bacterium *Streptomyces lividans*. It consists of four identical subunits (blue, yellow, green, and red), each of which contains two long α-helices and one shorter one. In the interior of the cell (bottom), the K^+ ions (violet) enter the structure's central channel. Before they are released to the outside, they have to pass through what is known as a "selectivity filter." In this part of the channel, several C=O groups in the peptide chain form a precisely defined opening that is only permeable to non-hydrated K^+ ions.



Membrane receptors

To receive and pass on chemical or physical signals, cells are equipped with **receptor proteins**. Many of these are integral membrane proteins in the plasma membrane, where they receive signals from their surroundings. Other receptor proteins are located in intercellular membranes. The receptors for lipophilic hormones are among the few that function in a soluble form. They regulate gene transcription in the nucleus (see p. 378).

A. Principle of receptor action ●

Membrane-located receptors can be divided into three parts, which have different tasks. The **receptor domain** reacts specifically to a given signal. Signals of this type can be of a purely physical nature. For example, many organisms react to light. In this way, plants adapt growth and photosynthesis to light conditions, while animals need light receptors for visual processing (**C**; see p. 358). Mechanoreceptors are involved in hearing and in pressure regulation, among other things. Channels that react to action potentials (see p. 350) can be regarded as receptors for electrical impulses.

However, most receptors do not react to physical stimuli, but rather to signal molecules. Receptors for these chemical signals contain binding sites in the receptor domain that are complementary to each ligand. In this respect, they resemble enzymes (see p. 94). As the **effector domain** of the receptor is usually separated by a membrane, a mechanism for **signal transfer** between the domains is needed. Little is yet known regarding this. It is thought that conformation changes in the receptor protein play a decisive part. Some receptors dimerize after binding of the ligand, thereby bringing the effector domains of two molecules into contact (see p. 392).

The way in which the effector works differs from case to case. By binding or interconversion, many receptors activate special **mediator proteins**, which then trigger a signal cascade (signal transduction; see p. 384). Other receptors function as **ion channels**. This is particularly widespread in receptors for neurotransmitters (see p. 354).

B. Insulin receptor ◑

The receptor for the hormone insulin (see p. 76) belongs to the family of **1-helix receptors**.

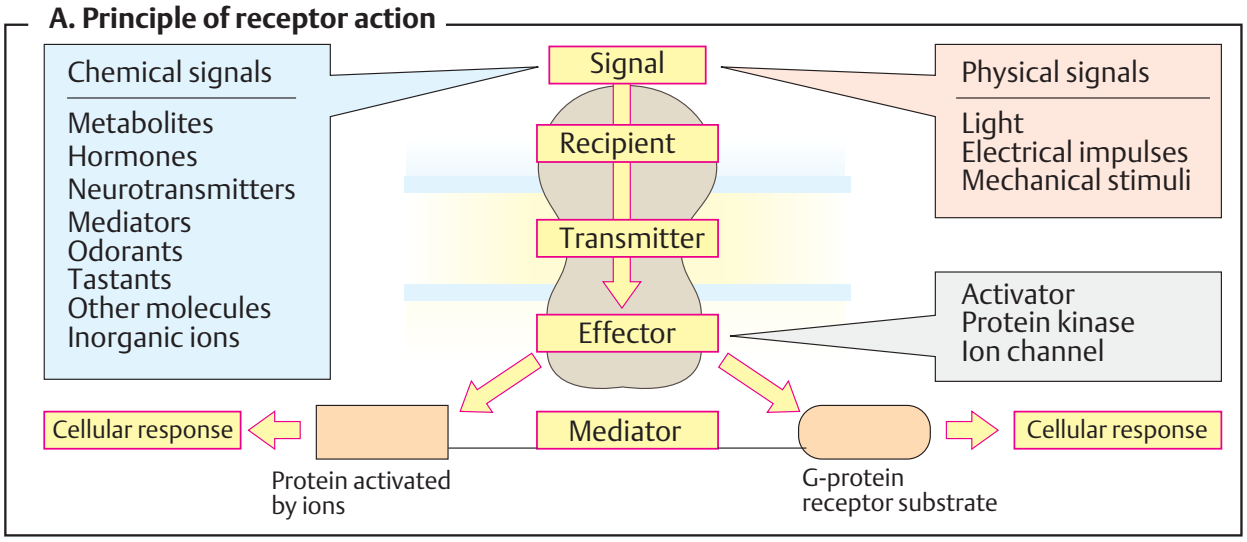
These molecules span the membrane with only one α-helix. The subunits of the dimeric receptor (red and blue) each consist of two polypeptides (α and β) bound by disulfide bonds. The α-chains together bind the insulin, while the β-chains contain the transmembrane helix and, at the C-terminus, domains with **tyrosine kinase** activity. In the activated state, the kinase domains phosphorylate themselves and also mediator proteins (receptor substrates) that set in motion cascades of further phosphorylations (see pp. 120 and 388).

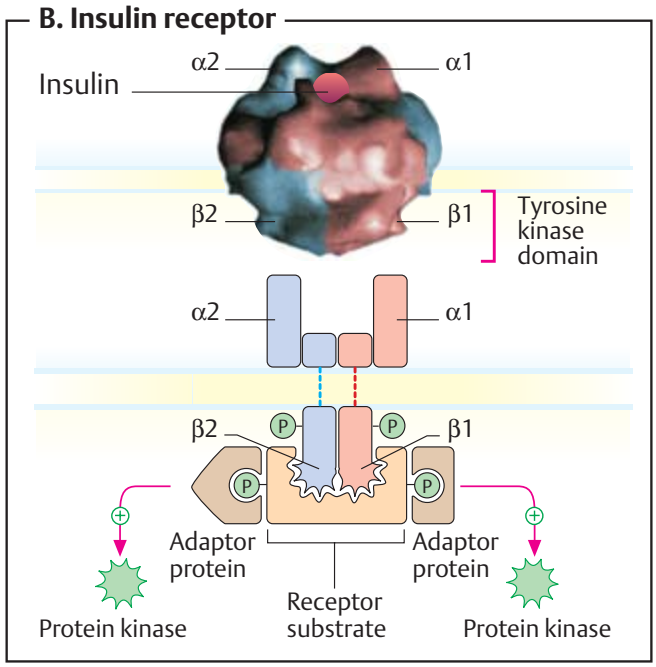
C. 7-helix receptors ◑

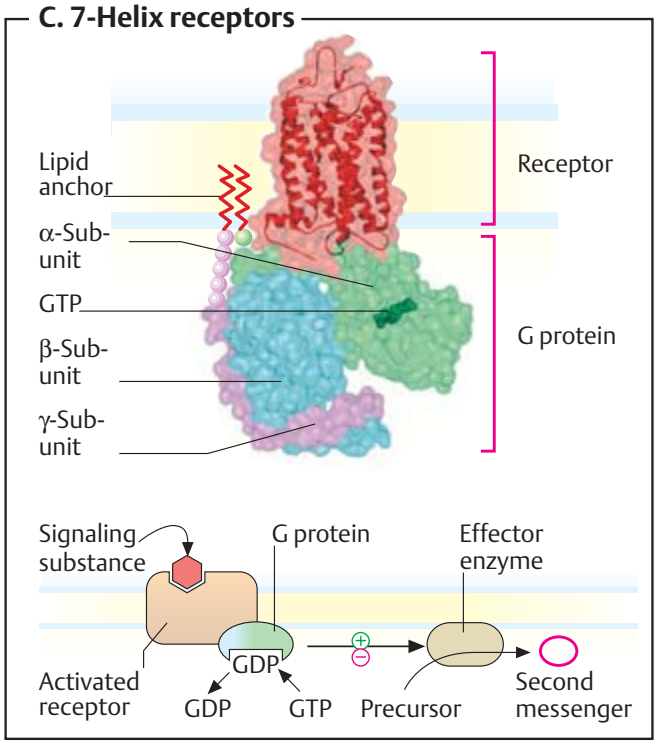
A large group of receptors span the membrane with α-helices seven times. These are known as **7-helix receptors**. Via their effector domains, they bind and activate trimeric proteins, which in turn bind and hydrolyze GTP and are therefore called G proteins. Most G proteins, in turn, activate or inhibit enzymes that create secondary signaling molecules (**second messengers**; see p. 386). Other G proteins regulate ion channels. The illustration shows the complex of the light receptor **rhodopsin**, with the associated G protein **transducin** (see p. 358). The GTP-binding α-subunit (green) and the γ-subunit (violet) of transducin are anchored in the membrane via lipids (see p. 214). The β-subunit is shown in detail on p. 72.

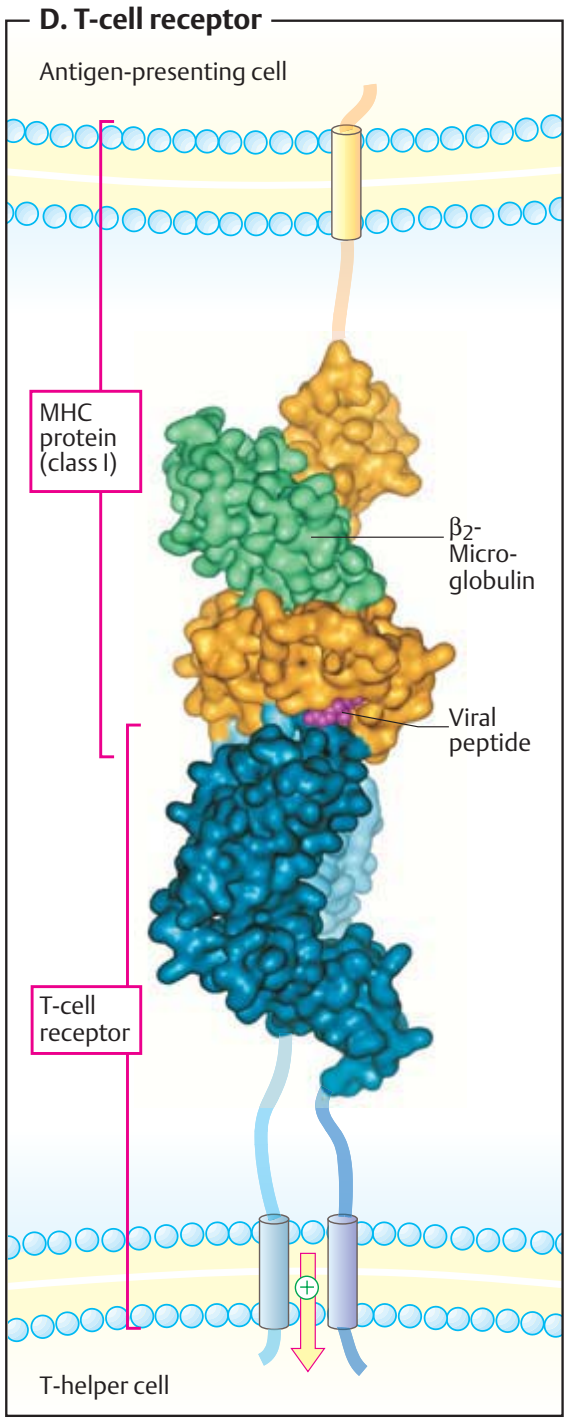
D. T-cell receptor ◑

The cells of the immune system communicate with each other particularly intensively. The **T-cell receptor** plays a central role in the activation of T lymphocytes (see p. 296). The cell at the top has been infected with a virus, and it indicates this by presenting a viral peptide (violet) with the help of a class I **MHC protein** (yellow and green). The combination of the two molecules is recognized by the dimeric T-cell receptor (blue) and converted into a signal that activates the T cell (bottom) and thereby enhances the immune response to the virus.









ER: structure and function

The endoplasmic reticulum (ER) is an extensive closed membrane system consisting of tubular and saccular structures. In the area of the nucleus, the ER turns into the external nuclear membrane. Morphologically, a distinction is made between the **rough ER** (rER) and the **smooth ER** (sER). Large numbers of ribosomes are found on the membranes of the rER, which are lacking on the sER. On the other hand, the sER is rich in membrane-bound **enzymes**, which catalyze partial reactions in the lipid metabolism as well as biotransformations.

A. Rough endoplasmic reticulum and the Golgi apparatus ◑

The **rER** (**1**) is a site of active *protein biosynthesis.* This is where proteins destined for membranes, lysosomes, and export from the cell are synthesized. The remaining proteins are produced in the cytoplasm on ribosomes that are not bound to membranes.

Proteins synthesized at the rER (**1**) are folded and modified after translation (protein maturation; see p. 230). They remain either in the rER as membrane proteins, or pass with the help of transport vesicles (**2**) to the Golgi apparatus (**3**). Transport vesicles are formed by budding from existing membranes, and they disappear again by fusing with them (see p. 228).

The **Golgi apparatus** (**3**) is a complex network, also enclosed, consisting of flattened membrane saccules ("cisterns"), which are stacked on top of each other in layers. Proteins mature here and are sorted and packed. A distinction is made between the *cis, medial,* and *trans* Golgi regions, as well as a *trans* Golgi network (tGN). The *post–translational modification of proteins,* which starts in the ER, continues in these sections.

From the Golgi apparatus, the proteins are transported by vesicles to various targets in the cells—e.g., to lysosomes (**4**), the plasma membrane (**6**), and secretory vesicles (**5**) that release their contents into the extracellular space by fusion with the plasma membrane (**exocytosis**; see p. 228). Protein transport can either proceed continuously (*constitutive*), or it can be *regulated* by chemical signals. The decision regarding which pathway a protein will take and whether its transport will be constitutive or regulated depends on the signal sequences or signal structures that proteins carry with them like address labels (see p. 228). In addition to proteins, the Golgi apparatus also transports membrane lipids to their targets.

B. Smooth endoplasmic reticulum ◑

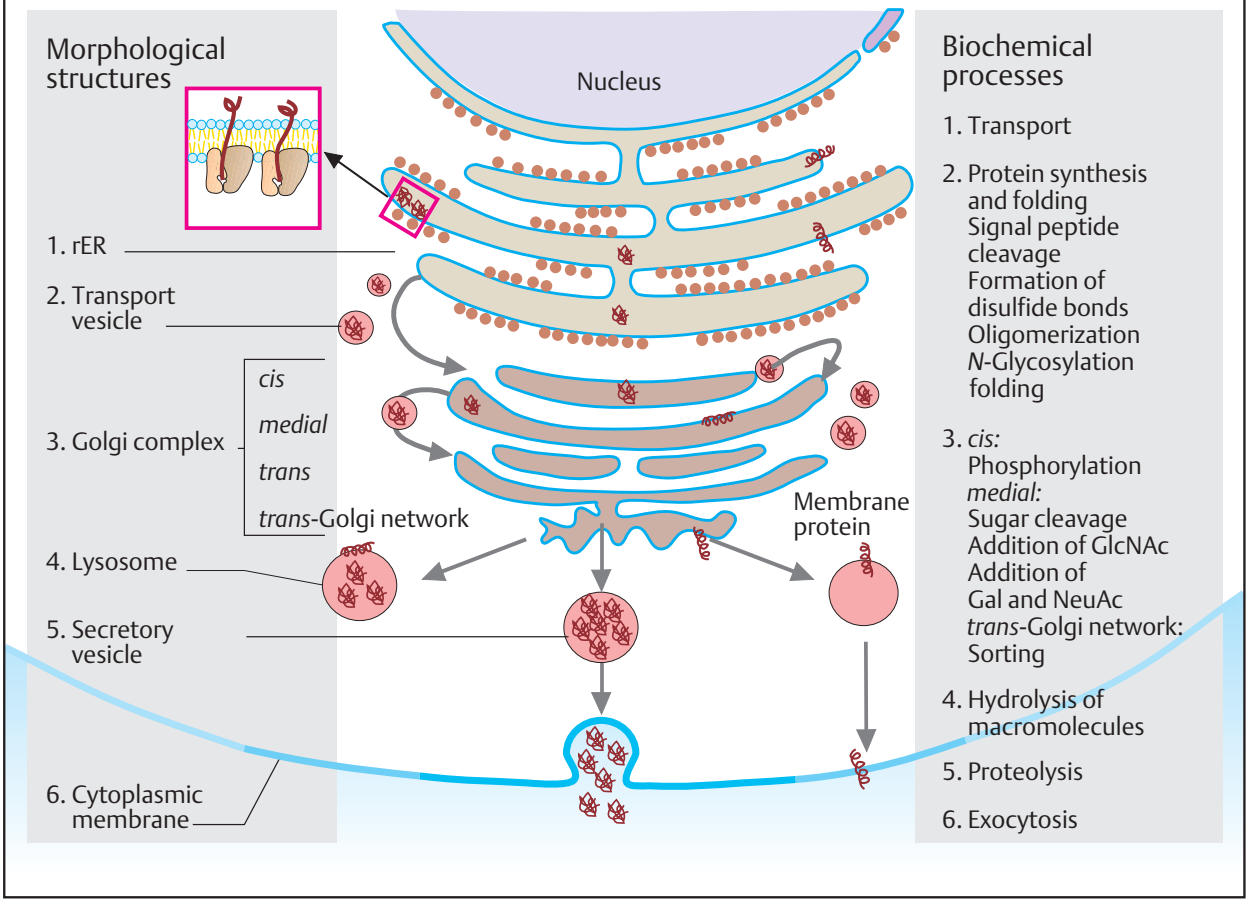
Regions of the ER that have no bound ribosomes are known as the **smooth endoplasmic reticulum** (sER). In most cells, the proportion represented by the sER is small. A marked sER is seen in cells that have an active lipid metabolism, such as hepatocytes and Leydig cells. The sER is usually made up of branching, closed tubules.

Membrane-located enzymes in the sER catalyze **lipid synthesis**. Phospholipid synthesis (see p. 170) is located in the sER, for example, and several steps in cholesterol biosynthesis (see p. 172) also take place there. In endocrine cells that form *steroid hormones,* a large proportion of the reaction steps involved also take place in the sER (see p. 376).

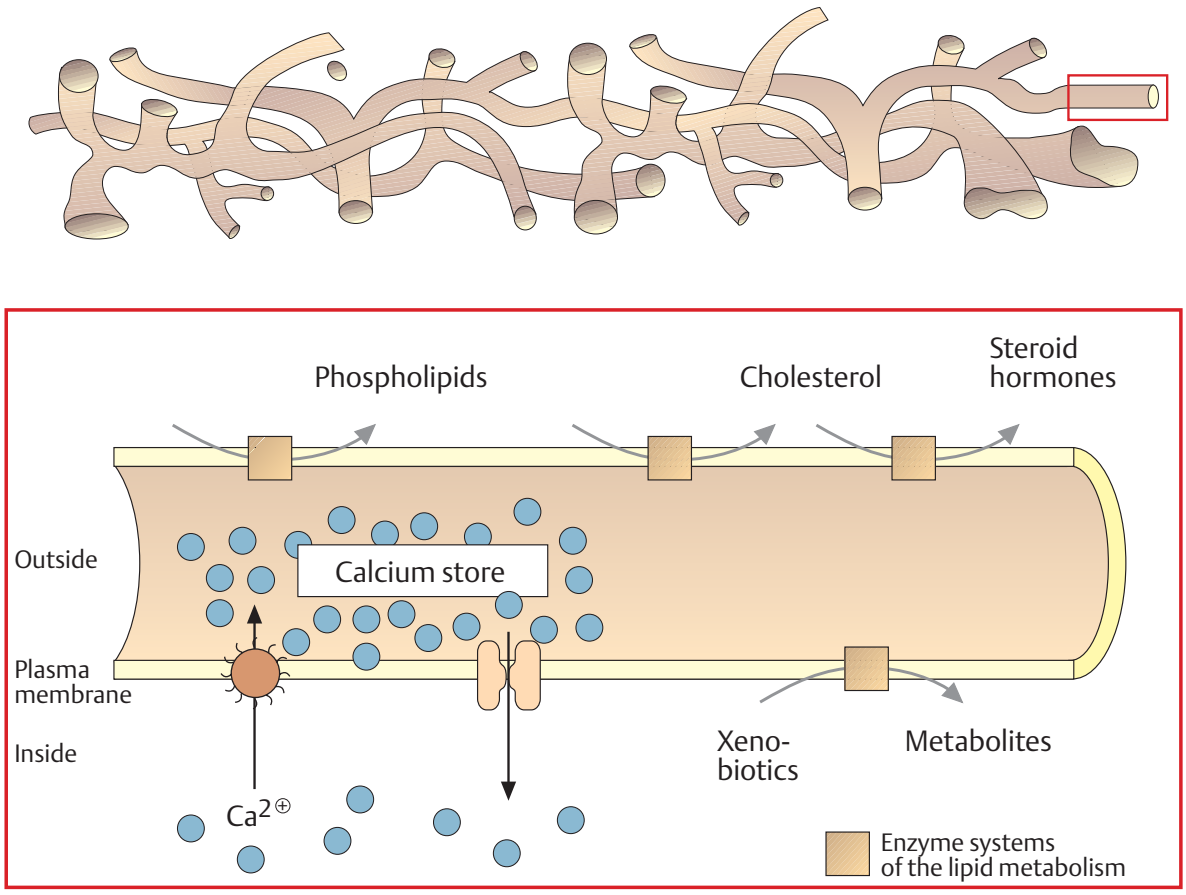
In the liver's hepatocytes, the proportion represented by the sER is particularly high. It contains enzymes that catalyze so-called **biotransformations**. These are reactions in which apolar foreign substances, as well as endogenous substances—e.g., steroid hormones—are chemically altered in order to inactivate them and/or prepare them for conjugation with polar substances (phase I reactions; see p. 316). Numerous *cytochrome P450 enzymes* are involved in these conversions (see p. 318) and can therefore be regarded as the major molecules of the sER.

The sER also functions as an intracellular **calcium store**, which normally keeps the Ca^{2+} level in the cytoplasm low. This function is particularly marked in the *sarcoplasmic reticulum*, a specialized form of the sER in muscle cells (see p. 334). For release and uptake of Ca^{2+}, the membranes of the sER contain signal-controlled Ca^{2+} channels and energy-dependent Ca^{2+} ATPases (see p. 220). In the lumen of the sER, the high Ca^{2+} concentration is buffered by Ca^{2+}-binding proteins.

A. Rough endoplasmic reticulum and Golgi apparatus



B. Smooth endoplasmic reticulum



Protein sorting

A. Protein sorting ◑

The biosynthesis of all proteins starts on free ribosomes (top). However, the paths that the proteins follow soon diverge, depending on which target they are destined for. Proteins that carry a *signal peptide for the ER* (**1**) follow the *secretory pathway* (right). Proteins that do not have this signal follow the *cytoplasmic pathway* (left).

Secretory pathway. Ribosomes that synthesize a protein with a signal peptide for the ER settle on the ER (see p. 228). The peptide chain is transferred into the lumen of the rER. The presence or absence of other signal sequences and signal regions determines the subsequent transport pathway.

Proteins that have *stop-transfer sequences* (**4**) remain as integral membrane proteins in the ER membrane. They then pass into other membranes via vesicular transport (see p. 226). From the rER, their pathway then leads to the Golgi apparatus and then on to the plasma membrane. Proteins destined to remain in the rER—e. g., enzymes—find their way back from the Golgi apparatus to the rER with the help of a *retention signal* (**2**). Other proteins move from the Golgi apparatus to the lysosomes (**3**; see p. 234), to the cell membrane (integral membrane proteins or constitutive exocytosis), or are transported out of the cell (**9;** signal-regulated exocytosis) by secretory vesicles (**8**).

Cytoplasmic pathway. Proteins that do not have a signal peptide for the ER are synthesized in the cytoplasm on free ribosomes, and remain in that compartment. Special signals mediate further transport into the mitochondria (**5**; see p. 232), the nucleus (**6**; see p. 208) or peroxisomes (**7**).

B. Translocation signals ○

Signal peptides are short sections at the N or C terminus, or within the peptide chain. Areas on the protein surface that are formed by various sections of the chain or by various chains are known as **signal regions**. Signal peptides and signal regions are *structural signals* that are usually recognized by *receptors* on organelles (see **A**). They move the proteins, with the help of additional proteins, into the organelles (*selective protein transfer*). Structural signals can also activate *enzymes* that modify the proteins and thereby determine their subsequent fate. Examples include lysosomal proteins (see p. 234) and membrane proteins with lipid anchors (see p. 214).

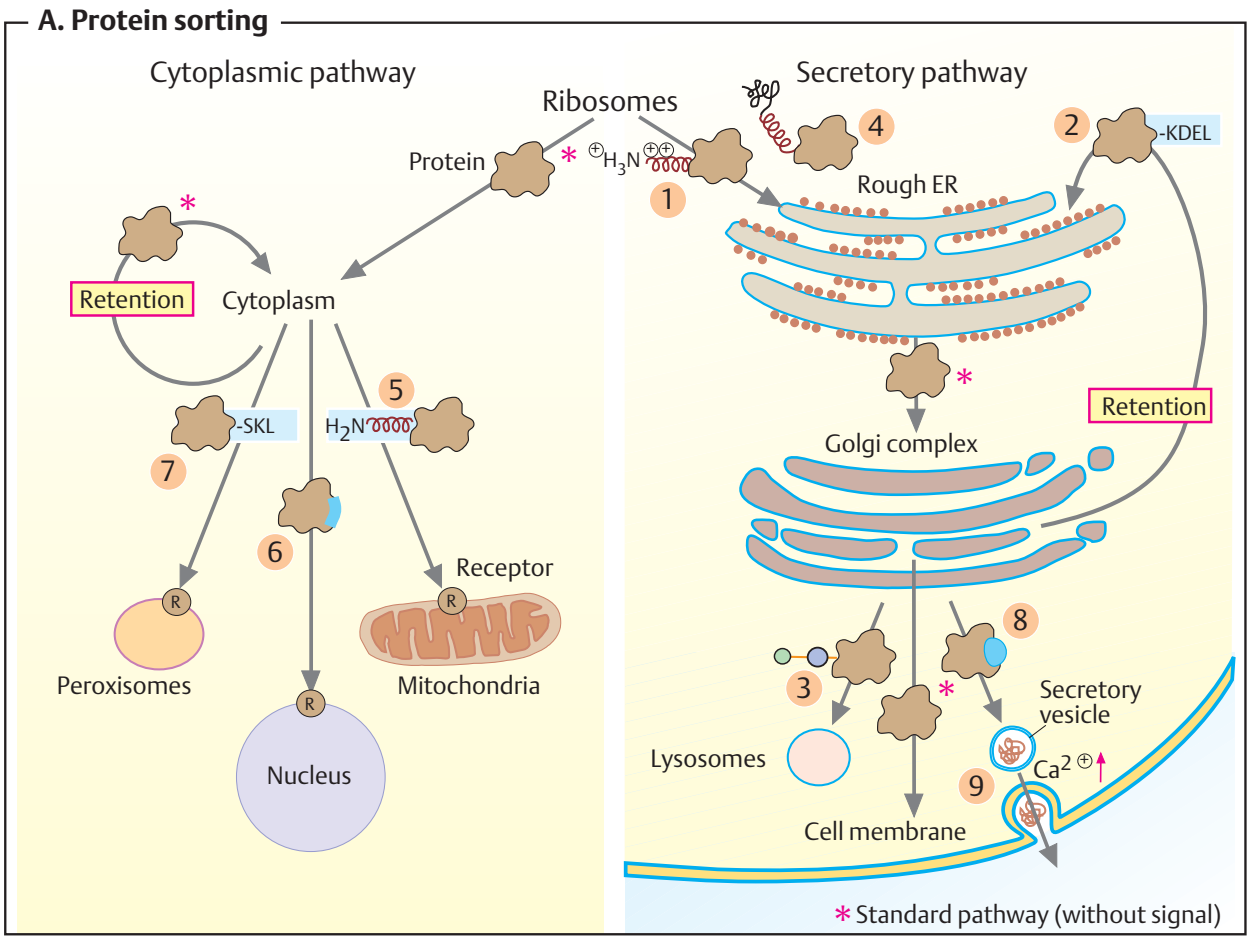
After they have been used, signal peptides at the N terminus are cleaved off by specific hydrolases (symbol: scissors). In proteins that contain several successive signal sequences, this process can expose the subsequent signals. By contrast, signal peptides that have to be read several times are not cleaved.

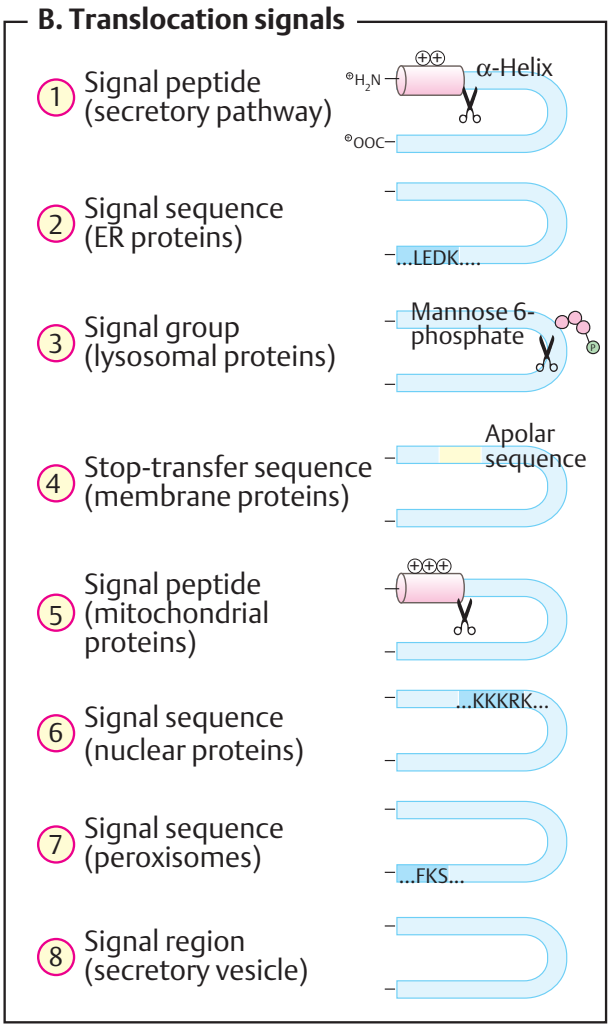
C. Exocytosis ○

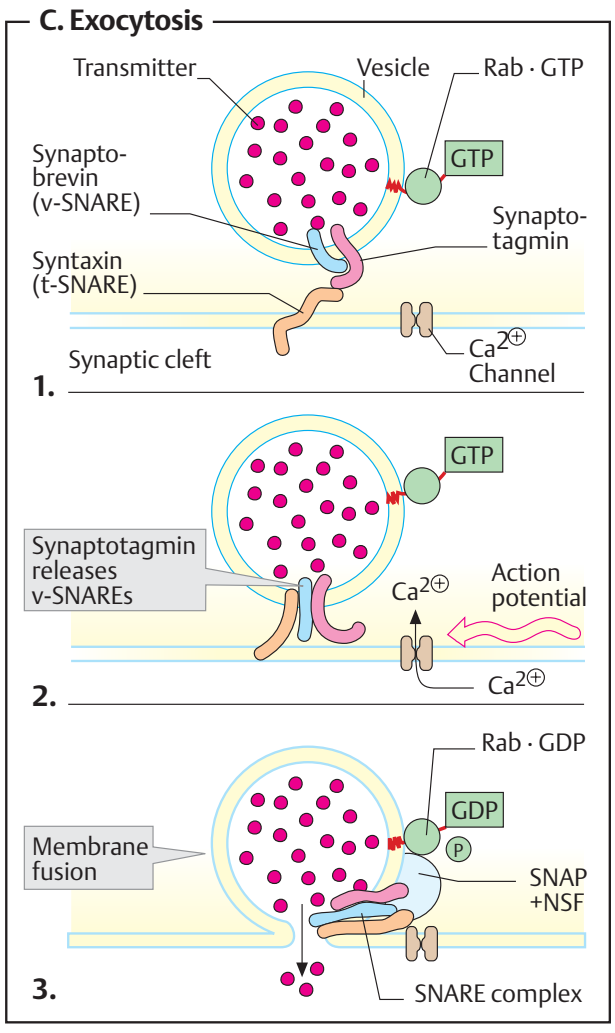
Exocytosis is a term referring to processes that allow cells to expel substances (e. g., hormones or neurotransmitters) quickly and in large quantities. Using a complex protein machinery, secretory vesicles fuse completely or partially with the plasma membrane and release their contents. Exocytosis is usually *regulated* by chemical or electrical signals. As an example, the mechanism by which neurotransmitters are released from synapses (see p. 348) is shown here, although only the most important proteins are indicated.

The decisive element in exocytosis is the interaction between proteins known as SNAREs that are located on the vesicular membrane (v-SNAREs) and on the plasma membrane (t-SNAREs). In the resting state (**1**), the v-SNARE *synaptobrevin* is blocked by the vesicular protein *synaptotagmin.* When an action potential reaches the presynaptic membrane, voltage-gated Ca^{2+} channels open (see p. 348). Ca^{2+} flows in and triggers the machinery by conformational changes in proteins. Contact takes place between synaptobrevin and the t-SNARE *synaptotaxin* (**2**). Additional proteins known as SNAPs bind to the SNARE complex and allow fusion between the vesicle and the plasma membrane (**3**). The process is supported by the hydrolysis of GTP by the auxiliary protein *Rab.*

The toxin of the bacterium *Clostridium botulinum,* one of the most poisonous substances there is, destroys components of the exocytosis machinery in synapses through enzymatic hydrolysis, and in this way blocks neurotransmission.







Protein synthesis and maturation

A. Protein synthesis in the rER ◑

With all proteins, protein biosynthesis (**Translation**; for details, see p. 250) starts on free ribosomes in the cytoplasm (**1**). Proteins that are exported out of the cell or into lysosomes, and membrane proteins of the ER and the plasma membrane, carry a **signal peptide for the ER** at their N-terminus. This is a section of 15–60 amino acids in which one or two strongly basic residues (Lys, Arg) near the N-terminus are followed by a strongly hydrophobic sequence of 10–15 residues (see p. 228).

As soon as the signal peptide (red) appears on the surface of the ribosome (**2**), an RNA-containing *signal recognition particle* (**SRP**, green) binds to the sequence and initially interrupts translation (**3**). The SRP then binds to an **SRP receptor** in the rER membrane, and in this way attaches the ribosome to the ER (**4**). After this, the SRP dissociates from the signal peptide and from the SRP receptor and is available again for step 3. This endergonic process is driven by GTP hydrolysis (**5**). Translation now resumes. The remainder of the protein, still unfolded, is gradually introduced into a channel (the **translocon**) in the lumen of the rER (**6**), where a *signal peptidase* located in the inner ER membrane cleaves the signal peptide while translation is still taking place (**7**). This converts the **preprotein** into a **proprotein**, from which the mature protein finally arises after additional post-translational modifications (**8**) in the ER and in the Golgi apparatus.

If the growing polypeptide contains a **stop-transfer signal** (see p. 228), then this hydrophobic section of the chain remains stuck in the membrane outside the translocon, and an *integral membrane protein* arises. In the course of translation, an additional signal sequence can re-start the transfer of the chain through the translocon. Several repetitions of this process produce integral membrane proteins with several transmembrane helices (see p. 214).

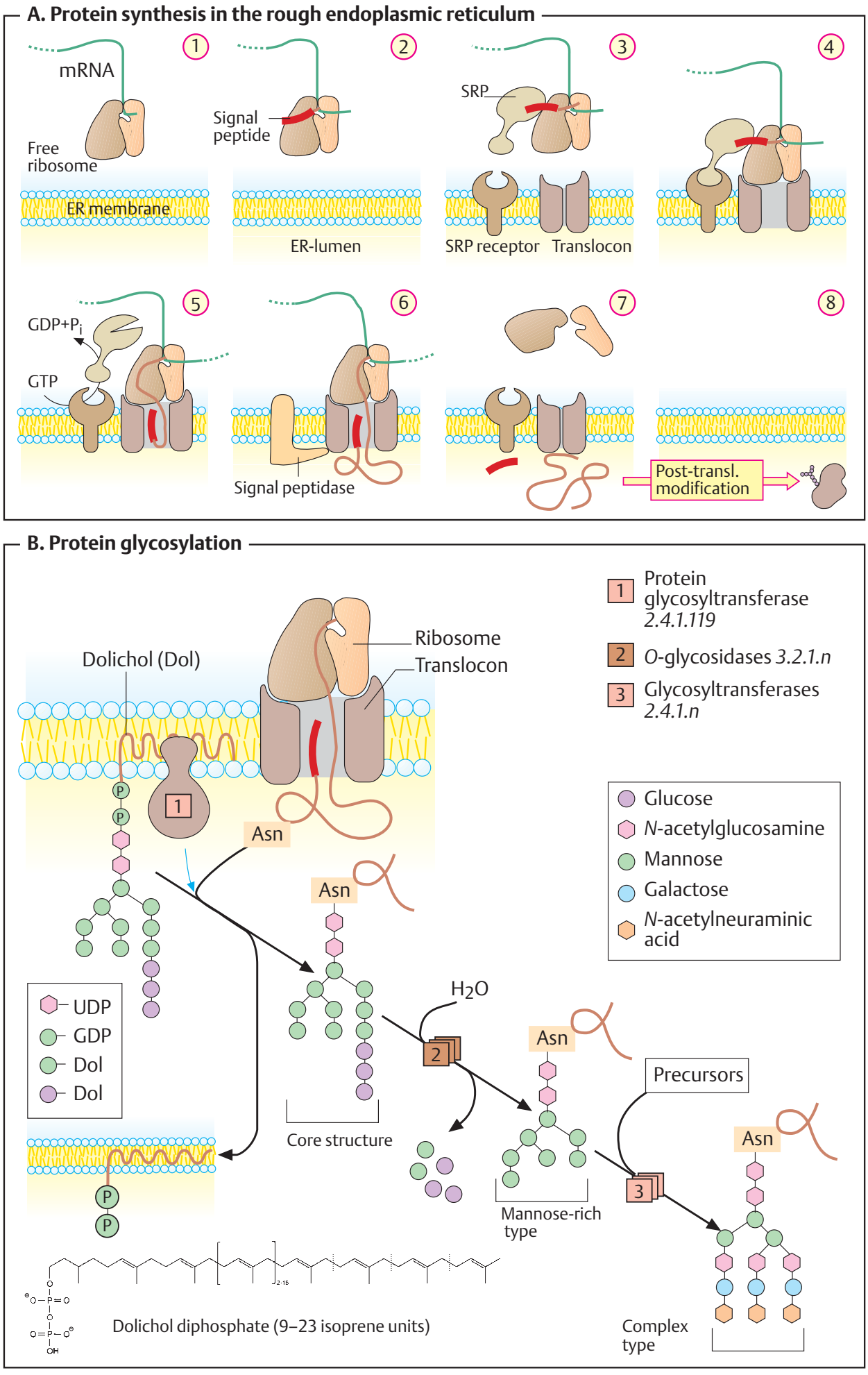
B. Protein glycosylation ○

Most extracellular proteins contain covalently bound oligosaccharide residues. For example, all plasma proteins with the exception of albumin are glycosylated. Together with glycolipids, numerous **glycoproteins** on the cell surface form the **glycocalyx**. Inside the ER, the carbohydrate parts of the glycoproteins are cotranslationally transferred to the growing chain, and are then converted into their final form while passing through the ER and Golgi apparatus.

N-bound oligosaccharides (see p. 44) are always bound to the acid-amide group of asparagine residues. If a **glycosylation sequence** (–Asn–X–Ser(Thr)–, where X can be any amino acid) appears in the growing peptide chain, then a *transglycosylase* in the ER membrane [1] transfers a previously produced **core oligosaccharide** consisting of 14 hexose residues *en-bloc* from the carrier molecule **dolichol diphosphate** to the peptide.

Dolichol is a long-chain isoprenoid (see p. 52) consisting of 10–20 isoprene units, which is embedded in the ER membrane. A hydroxyl group at the end of the molecule is bound to diphosphate, on which the nuclear oligosaccharide is built up in an extended reaction sequence (not shown here in detail). The core structure consists of two residues of N-acetylglucosamine (GlcNAc), a branched group of nine mannose residues (Man) and three terminal glucose resides (Glc).

As the proprotein passes through the ER, *glycosidases* [2] remove the glucose residues completely and the mannoses partially ("trimming"), thereby producing the **mannose-rich type** of oligosaccharide residues. Subsequently, various *glycosyltransferases* [3] transfer additional monosaccharides (e.g., GlcNAc, galactose, fucose, and N-acetylneuraminic acid; see p. 38) to the mannose-rich intermediate and thereby produce the **complex type** of oligosaccharide. The structure of the final oligosaccharide depends on the type and activity of the glycosyltransferases present in the ER of the cell concerned, and is therefore genetically determined (although indirectly).



Protein maturation

After translation, proteins destined for the secretory pathway (see p. 228) first have to fold into their native conformation within the rER (see p. 230). During this process they are supported by various *auxiliary proteins.*

A. Protein folding in the rER ●

To prevent incorrect folding of the growing protein during protein biosynthesis, **chaperones** (see **B**) in the lumen of the rER bind to the peptide chain and stabilize it until translation has been completed. Binding protein (**BiP**) is an important chaperone in the ER.

Many secretory proteins—e. g., pancreatic ribonuclease (RNAse; see p. 74)—contain several disulfide bonds that are only formed oxidatively from SH groups after translation. The eight cysteine residues of the RNAse can in principle form 105 different pairings, but only the combination of the four disulfide bonds shown on p. 75 provides active enzyme. Incorrect pairings can block further folding or lead to unstable or insoluble conformations. The enzyme *protein disulfide isomerase* [1] accelerates the equilibration between paired and unpaired cysteine residues, so that incorrect pairs can be quickly split before the protein finds its final conformation.

Most peptide bonds in proteins take on the *trans* conformation (see p. 66). Only bonds with proline residues (–X–Pro–) can be present in both *cis* and *trans* forms.

In the protein's native conformation, every X–Pro bond has to have the correct conformation (*cis* or *trans*). As the uncatalyzed transition between the two forms is very slow, there is a *proline* cis–trans *isomerase* [2] in the ER that accelerates the conversion.

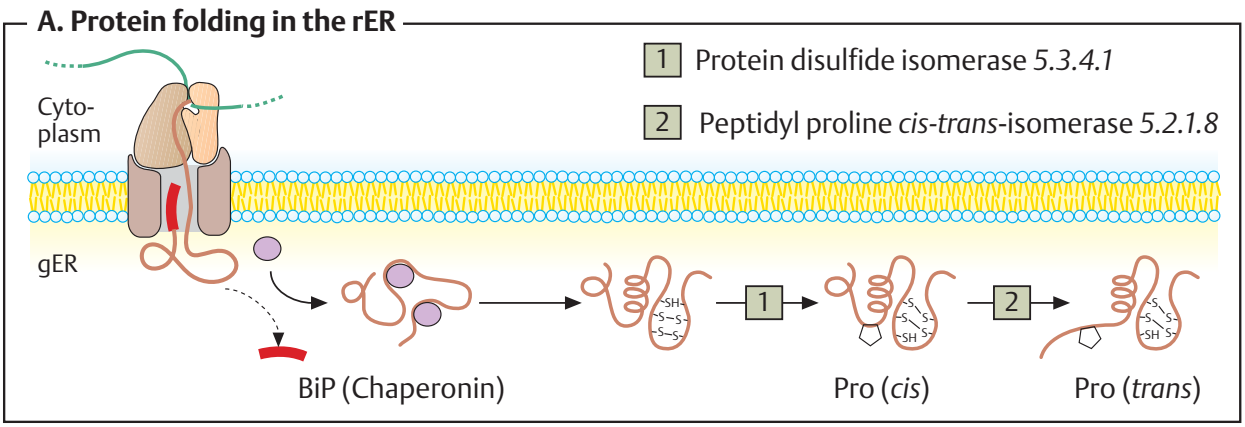
B. Chaperones and chaperonins ●

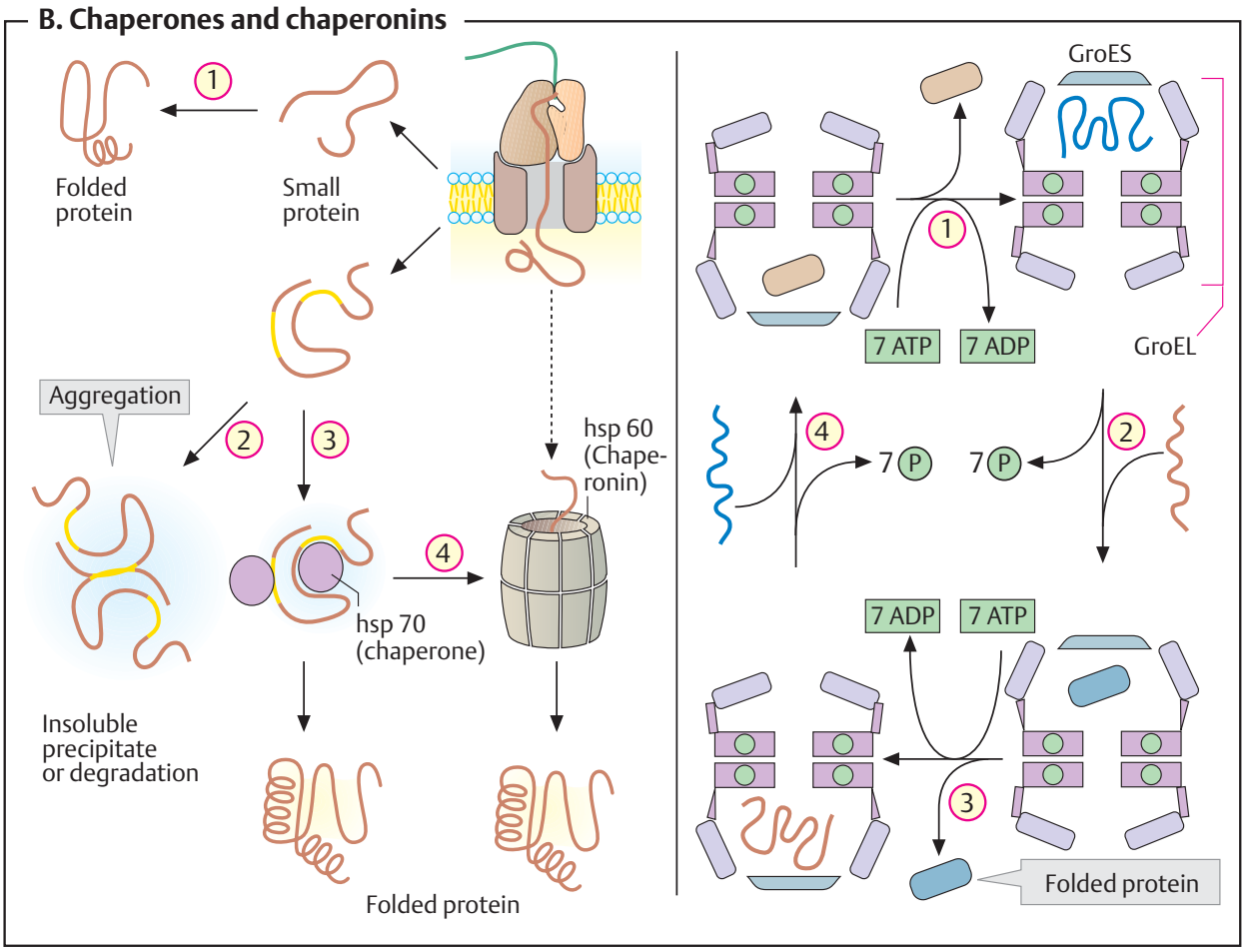
Most proteins fold spontaneously into their native conformation, even in the test tube. In the cell, where there are very high concentrations of proteins (around 350 g L^{-1}), this is more dif cult. In the unfolded state, the apolar regions of the peptide chain (yellow) tend to aggregate—due to the hydrophobic effect (see p. 28)—with other proteins or with each other to form insoluble products (2). In addition, unfolded proteins are susceptible to proteinases. To protect partly folded proteins, there are auxiliary proteins called **chaperones** because they guard immature proteins against damaging contacts. Chaperones are formed increasingly during temperature stress and are therefore also known as **heat-shock proteins** (hsp). Several classes of hsp are distinguished. Chaperones of the hsp70 type (Dna K in bacteria) are common, as are type hsp60 **chaperonins** (GroEL/ES in bacteria). Class hsp90 chaperones have special tasks (see p. 378).

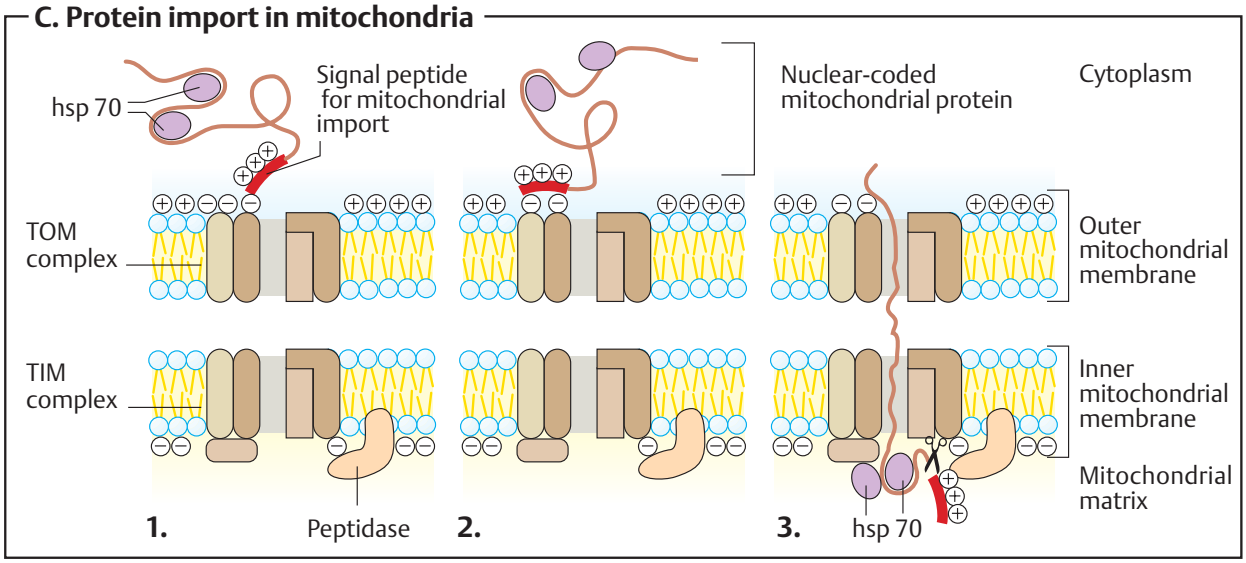
While small proteins can often reach their native conformation without any help (1), larger molecules require hsp70 proteins for protection against aggregation which bind as monomers and can dissociate again, dependent on ATP (3). By contrast, type hsp60 chaperonins form large, barrel-shaped complexes with 14 subunits in which proteins can fold independently while shielded from their environment (4). The function of hsp60 has been investigated in detail in the bacterial chaperonin **GroEL** (right). The barrel has two chambers, which are closed with a lid (**GroES**) during folding of the guest protein. Driven by ATP hydrolysis, the chambers open and close alternately—i. e., the release of the fully folded protein from one chamber is coupled to the uptake of an unfolded peptide in the second chamber.

C. Protein import in mitochondria ●

Class hsp70 chaperones are also needed for translocation of nuclear-coded proteins from the cytoplasm into the mitochondria (see p. 228). As two membranes have to be crossed to reach the matrix, there are two translocator complexes: **TOM (**"transport outer membrane") and **TIM** ("transport inner membrane"). For transport, proteins are unfolded in the cytoplasm and protected by hsp70. TOM recognizes the positively charged signal sequence at the protein's N terminus (see p. 228) and with the help of the membrane potential threads the chains through the central pores of the two complexes. Inside TIM, further hsp70 molecules bind and pull the chain completely into the matrix. As with import into the ER, the signal peptide is proteolytically removed by a signal peptidase during translocation.







Lysosomes

A. Structure and contents ◑

Animal lysosomes are organelles with a diameter of 0.2–2.0 μm with various shapes that are surrounded by a single membrane. There are usually several hundred lysosomes per cell. ATP-driven V-type proton pumps are active in their membranes (see p. 220). As these accumulate H^+ in the lysosomes, the content of lysosomes with pH values of 4.5–5 is much more acidic than the cytoplasm (pH 7–7.3).

The lysosomes are the cell's "stomach," serving to break down various cell components. For this purpose, they contain some 40 different types of **hydrolases**, which are capable of breaking down every type of macromolecule. The marker enzyme of lysosomes is *acid phosphatase*. The pH optimum of lysosomal enzymes is adjusted to the acid pH value and is also in the range of pH 5. At neutral pH, as in the cytoplasm, lysosomal enzymes only have low levels of activity. This appears to be a mechanism for protecting the cells from digesting themselves in case lysosomal enzymes enter the cytoplasm at any time. In plants and fungi, the **cell vacuoles** (see p. 43) have the function of lysosomes.

B. Functions ◑

Lysosomes serve for enzymatic degradation of macromolecules and cell organelles, which are supplied in various ways. The example shows the degradation of an overaged mitochondrion by *autophagy*. To accomplish this, the lysosome encloses the organelle (**1**). During this process, the **primary lysosome** converts into a **secondary lysosome**, in which the hydrolytic degradation takes place (**2**). Finally, **residual bodies** contain the indigestible residues of the lysosomal degradation process. Lysosomes are also responsible for the degradation of macromolecules and particles taken up by cells via *endocytosis* and *phagocytosis*—e. g., lipoproteins, proteohormones, and bacteria (*heterophagy*). In the process, lysosomes fuse with the **endosomes** (**3**) in which the endocytosed substances are supplied.

C. Synthesis and transport of lysosomal proteins ◑

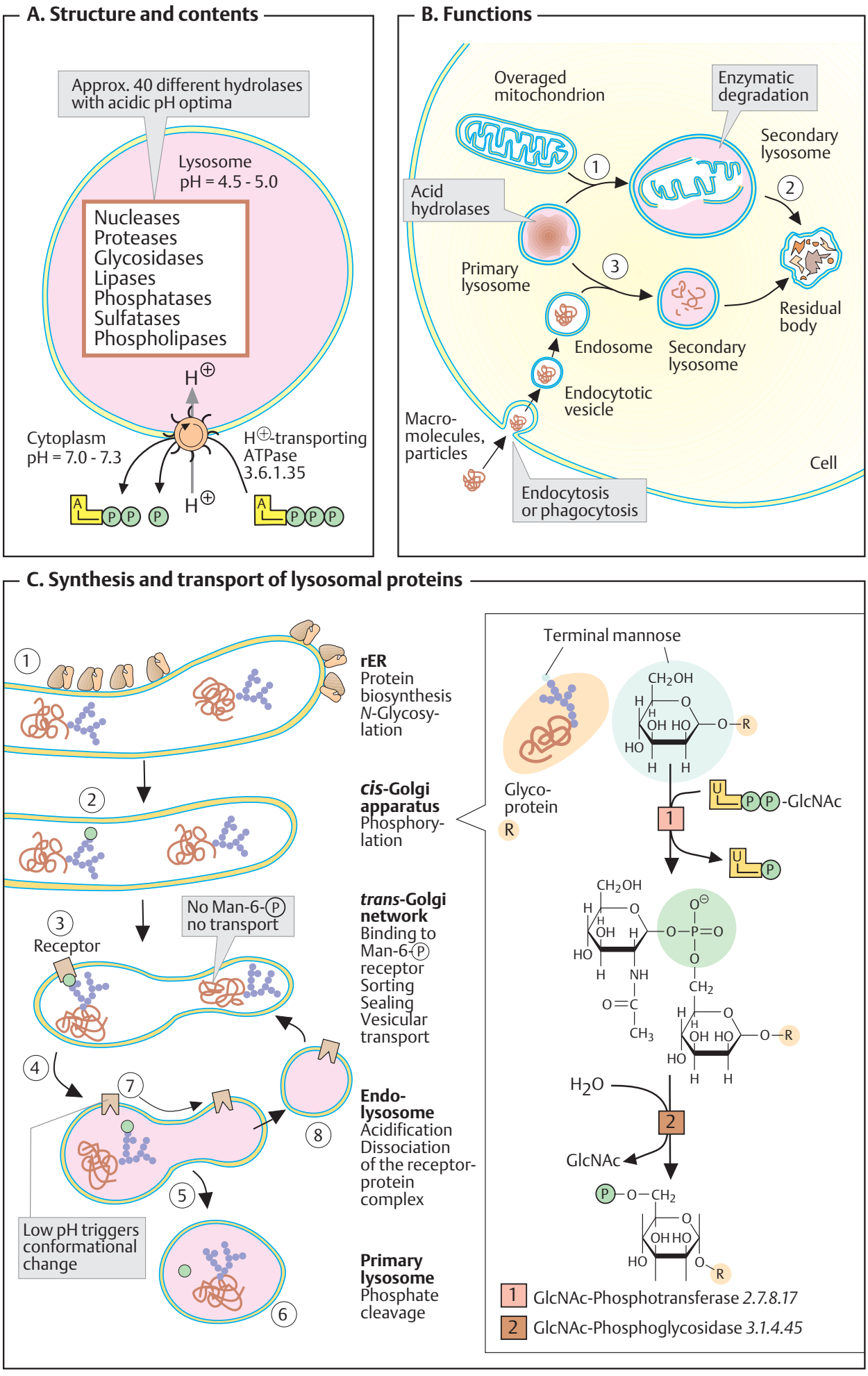
Primary lysosomes arise in the region of the Golgi apparatus. **Lysosomal proteins** are synthesized in the rER and are glycosylated there as usual (**1**; see p. 228). The next steps are specific for lysosomal proteins (right part of the illustration). In a two-step reaction, terminal mannose residues (Man) are phosphorylated at the C–6 position of the mannose. First, *N*-acetylglucosamine 1-phosphate is transferred to the OH group at C-6 in a terminal mannose residue, and *N*-acetylglucosamine is then cleaved again. Lysosomal proteins therefore carry a terminal **mannose 6–phosphate** (Man6–P; **2**).

The membranes of the Golgi apparatus contain receptor molecules that bind Man 6–P. They recognize lysosomal proproteins by this residue and bind them (**3**). With the help of *clathrin*, the receptors are concentrated locally. This allows the appropriate membrane sections to be pinched off and transported to the endolysosomes with the help of transport vesicles (**4**), from which primary lysosomes arise through maturation (**5**). Finally, the phosphate groups are removed from Man 6–P (**6**).

The *Man 6–P receptors* are reused. The fall in the pH value in the endolysosomes releases the receptors from the bound proteins (**7**) which are then transported back to the Golgi apparatus with the help of transport vesicles.

Further information

Many hereditary diseases are due to genetic defects in lysosomal enzymes. The metabolism of glycogen (→ *glycogenoses*), lipids (→ *lipidoses*), and proteoglycans (→ *mucopolysaccharidoses*) is particularly affected. As the lysosomal enzymes are indispensable for the intracellular breakdown of macromolecules, unmetabolized macromolecules or degradation products accumulate in the lysosomes in these diseases and lead to irreversible cell damage over time. In the longer term, enlargement takes place, and in severe cases there may be failure of the organ affected—e. g., the liver.



Molecular genetics: overview

Nucleic acids (**DNA** and various **RNAs**) are of central importance in the storage, transmission, and expression of genetic information. The decisive factor involved is their ability to enter into specific base pairings with each other (see p. 84). The individual processes involved, which are summed up in an overview here, are discussed in more detail on the following pages.

A. Expression and transmission of genetic information ●

Storage. The genetic information of all cells is stored in the base sequence of their DNA (RNA only occurs as a genetic material in viruses; see p. 404). Functional sections of DNA that code for inheritable structures or functions are referred to as **genes**. The 30 000–40 000 human genes represent only a few percent of the **genome**, which consists of approximately $5 \cdot 10^9$ base pairs (bp). Most genes code for proteins—i.e., they contain the information for the sequence of amino acid residues of a protein (its sequence). Every amino acid residue is represented in DNA by a code word (a **codon**) consisting of a sequence of three base pairs (a triplet). At the level of DNA, codons are defined as sequences of the sense strand read in the 5′→3′ direction (see p. 84). A DNA codon for the amino acid phenylalanine, for example, is thus *TTC* (**2**).

Replication. During cell division, all of the genetic information has to be passed on to the daughter cells. To achieve this, the whole of the DNA is copied during the S phase of the cell cycle (see p. 394). In this process, each strand serves as a matrix for the synthesis of a complementary second strand (**1**; see p. 240).

Transcription. For *expression* of a gene—i.e., synthesis of the coded protein—the DNA sequence information has to be converted into a protein sequence. As DNA itself is not involved in protein synthesis, the information is transferred from the nucleus to the site of synthesis in the cytoplasm. To achieve this, the *template strand* in the relevant part of the gene is transcribed into an **RNA** (hnRNA). The sequence of this RNA is thus complementary to that of the *template strand* (**3**), but—with the exception of the exchange of thymine for uracil—it is identical to that of the sense strand. In this way, the DNA triplet *TTC* gives rise in hnRNA to the RNA codon *UUC*.

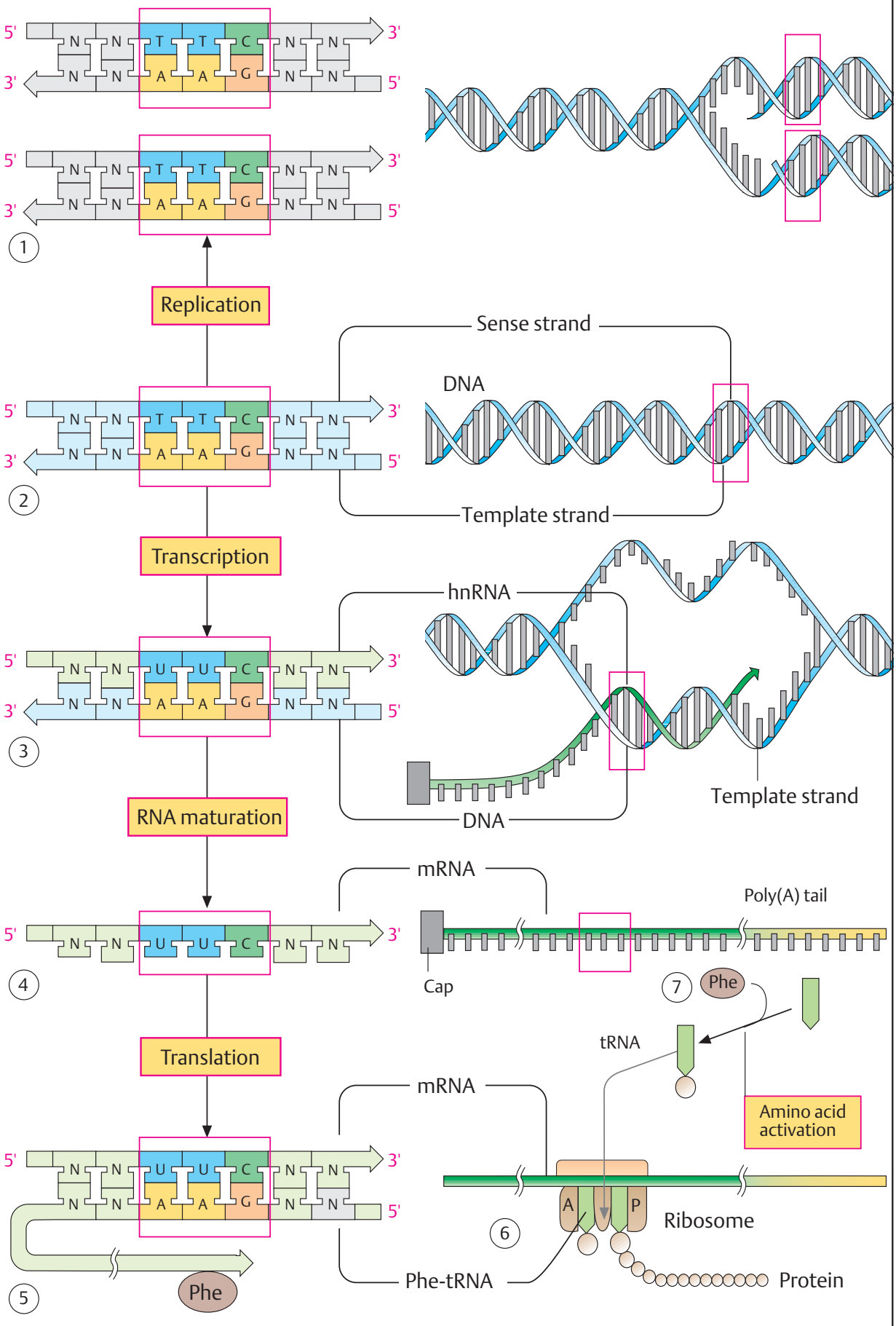
RNA maturation. In eukaryotes, the hnRNA initially formed is modified several times before it can leave the nucleus as **messenger RNA** (mRNA, **4**). During RNA maturation, superfluous ("intervening") sequences (introns) are removed from the molecule, and both ends of the transcript are protected by the addition of further nucleotides (see p. 246).

Translation. Mature mRNA enters the cytoplasm, where it binds to **ribosomes**, which convert the RNA information into a peptide sequence. The ribosomes (see p. 250) consist of more than 100 proteins and several RNA molecules (rRNA; see p. 82). rRNA plays a role as a ribosomal structural element and is also involved in the binding of mRNA to the ribosome and the formation of the peptide bond.

The actual information transfer is based on the interaction between the mRNA codons and another type of RNA, **transfer RNA** (tRNA; see p. 82). tRNAs, of which there are numerous types, always provide the correct amino acid to the ribosome according to the sequence information in the mRNA. tRNAs are loaded with an amino acid residue at the 3′ end. Approximately in the middle, they present the triplet that is complementary to each mRNA codon, known as the *anticodon* (*GAA* in the example shown). If the codon *UUC* appears on the mRNA, the anticodon binds a molecule of Phe-t-RNA^{Phe} to the mRNA (**5**) and thus brings the phenylalanine residue at the other end of the molecule into a position in which it can take over the growing polypeptide chain from the neighboring tRNA (**6**).

Amino acid activation. Before binding to the ribosomes, tRNAs are loaded with the correct amino acids by specific ligases (**7**; see p. 248). It is the amino acid tRNA ligases that carry out the transfer (translation) of the genetic information from the nucleic acid level to the protein level.

A. Expression and transmission of genetic information



Genome

A. Chromatin ◑

In the nuclei of eukaryotes (see p. 196), DNA is closely associated with proteins and RNA. These nucleoprotein complexes, with a DNA proportion of approximately one-third, are known as **chromatin**. It is only during cell division (see p. 394) that chromatin condenses into **chromosomes** that are visible under light microscopy. During interphase, most of the chromatin is loose, and in these conditions a morphological distinction can be made between tightly packed **heterochromatin** and the less dense **euchromatin**. Euchromatin is the site of active transcription.

The proteins contained in chromatin are classified as either histone or non-histone proteins. **Histones** (**B**) are small, strongly basic proteins that are directly associated with DNA. They contribute to the structural organization of chromatin, and their basic amino acids also neutralize the negatively charged phosphate groups, allowing the dense packing of DNA in the nucleus. This makes it possible for the 46 DNA molecules of the diploid human genome, with their 5 10^9 base pairs (bp) and a total length of about 2 m, to be accommodated in a nucleus with a diameter of only 10 μm. Histones also play a central role in regulating transcription (see p. 244).

Two histone molecules each of types **H2A** (blue), **H2B** (green), **H3** (yellow), and **H4** (red) form an octameric complex, around which 146 bp of DNA are wound in 1.8 turns. These particles, with a diameter of 7 nm, are referred to as **nucleosomes**. Another histone (**H1**) binds to DNA segments that are not directly in contact with the histone octamers ("linker" DNA). It covers about 20 bp and supports the formation of spirally wound superstructures with diameters of 30 nm, known as **solenoids**. When chromatin condenses into chromosomes, the solenoids form **loops** about 200 nm long, which already contain about 80 000 bp. The loops are bound to a protein framework (the **nuclear scaffolding**), which in turn organizes some 20 loops to form **minibands**. A large number of stacked minibands finally produces a chromosome. In the chromosome, the DNA is so densely packed that the smallest human chromosome already contains more than 50 million bp.

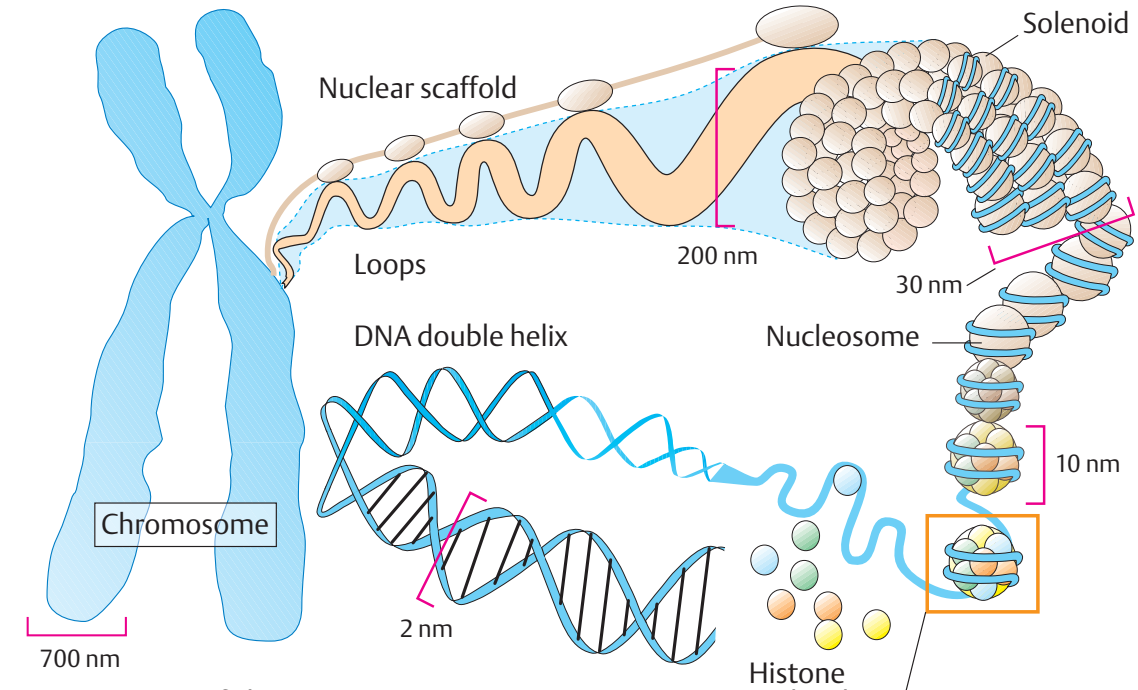
The **non-histone proteins** are very heterogeneous. This group includes *structural proteins* of the nucleus, as well as many *enzymes* and *transcription factors* (see p. 118), which selectively bind to specific segments of DNA and regulate gene expression and other processes.

B. Histones ○

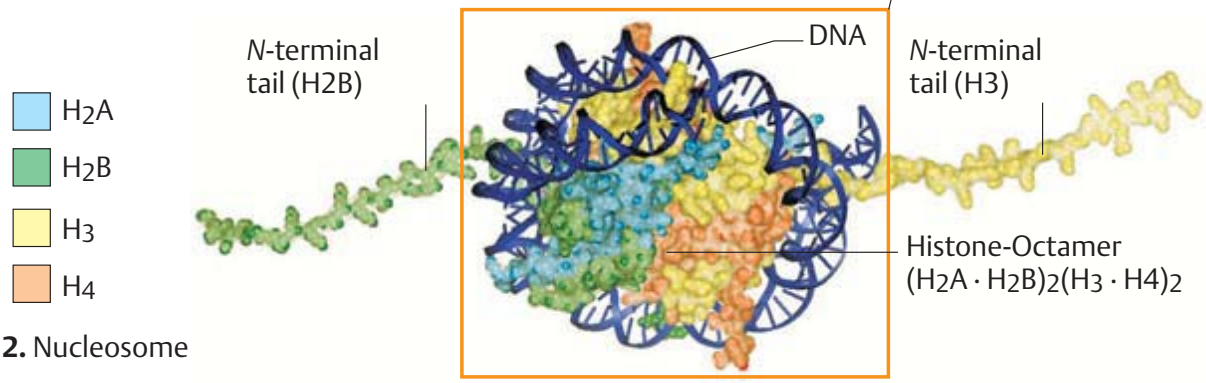
The histones are remarkable in several ways. With their high proportions of lysine and arginine (blue shading), they are strongly basic, as mentioned above. In addition, their amino acid sequence has hardly changed at all in the course of evolution. This becomes clear when one compares the histone sequences in mammals, plants, and fungi (yeasts are single-celled fungi; see p. 148). For example, the H4 histones in humans and wheat differ only in a single amino acid residue, and there are only a few changes between humans and yeast. In addition, all of these changes are "conservative"—i. e., the size and polarity barely differ. It can be concluded from this that the histones were already "optimized" when the last common predecessor of animals, plants, and fungi was alive on Earth (more than 700 million years ago). Although countless mutations in histone genes have taken place since, almost all of these evidently led to the extinction of the organisms concerned.

The histones in the octamer carry N-terminal mobile "tails" consisting of some 20 amino acid residues that project out of the nucleosomes and are important in the regulation of chromatin structure and in controlling gene expression (see **A2**; only two of the eight tails are shown in full length). For example, the condensation of chromatin into chromosomes is associated with *phosphorylation* (P) of the histones, while the transcription of genes is initiated by *acetylation* (A) of lysine residues in the N-terminal region (see p. 244).

A. Chromatin



1. Organization of chromatin



2. Nucleosome

B. Histone

A Acetylation P Phosphorylation M Methylation

	1	10	20	30	40	50	60	70	80	90	100
Animals	S G R G K G G K G L G K G G A K R H R K V L R D N I										
Plants	S G R G K G G K G L G K G G A K R H R K V L R D N I										
Yeast	S G R G K G G K G L G K G G A K R H R K I L R D N I										
Animals				Q G I T K P A I R R L A R R G G V K R I S G L I Y E							
Plants				Q G I T K P A I R R L A R R G G V K R I S G L I Y E							
Yeast				Q G I T K P A I R R L A R R G G V K R I S G L I Y E							
Animals							E T R G V L K V F L E N V I R D A V T Y T E H A K R				
Plants							E T R G V L K I F L E N V I R D A V T Y T E H A R R				
Yeast							E V R A V L K S F L E S V I R D S V T Y T E H A K R				
Animals									K T V T A M D V V Y A L K R Q G R T L Y G F G G		
Plants									K T V T A M D V V Y A L K R Q G R T L Y G F G G		
Yeast									K T V T S L D V V Y A L K R Q G R T L Y G F G G		

Amino acid sequence of histone H4

Replication

For genetic information to be passed on during cell division, a complete copy of the genome has to be produced before each mitosis. This process is known as DNA **replication**.

A. Mechanism of DNA polymerases ◑

Replication is catalyzed by *DNA–dependent DNA polymerases.* These enzymes require a single strand of DNA, known as the **template**. Beginning at a short starting sequence of RNA (the **primer**), they synthesize a second complementary strand on the basis of this template, and thus create a complete DNA double helix again. The substrates of the DNA polymerases are the four deoxynucleoside triphosphates **dATP**, **dGTP**, **dCTP**, and **dTTP**. In each step, base pairing first binds the nucleotide that is complementary to the current base in the template strand. The α–phosphate residue of the newly bound nucleoside triphosphate is then subjected to nucleophilic attack by the 3′–OH group of the nucleotide incorporated immediately previously. This is followed by the elimination of diphosphate and the formation of a new phosphoric acid diester bond. These steps are repeated again for each nucleotide. The mechanism described means that the matrix can only be read in the 3′→5′ direction. In other words, the newly synthesized strand always grows in the **5′→3′ direction**. The same mechanism is also used in transcription by *DNA-dependent RNA polymerases* (see p. 242). Most DNA and RNA polymerases consist of more than 10 subunits, the role of which is still unclear to some extent.

B. Replication in *E. coli* ◑

Although replication in prokaryotes is now well understood, many details in eukaryotes are still unclear. However, it is certain that the process is in principle similar. A simplified scheme of replication in the bacterium *Escherichia coli* is shown here.

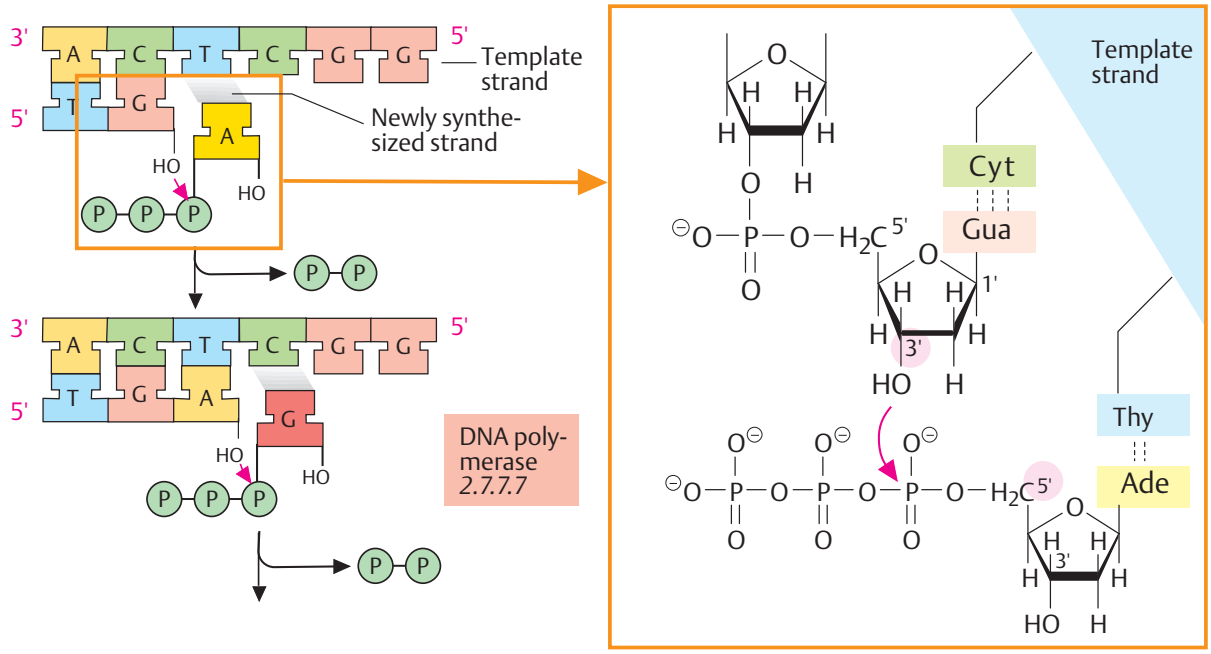
In bacteria, replication starts at a specific point in the circular DNA—the **origin of replication**—and proceeds in both directions. This results in two diverging **replication forks**, in which the two strands are replicated simultaneously. Numerous proteins are involved in the processes taking place in this type of fork, and only the most important are shown here. The two strands of the initial DNA (**1**) are shown in blue and violet, while the newly formed strands are pink and orange.

Each fork (**2**) contains two molecules of **DNA polymerase III** and a number of helper proteins. The latter include **DNA topoisomerases** and **single–strand-binding proteins**. Topoisomerases are enzymes that unwind the superhelical DNA double strand (*gyrase, topoisomerase II*) and then separate it into the two individual strands (*helicase, topoisomerase I*). Since the template strand is always read from 3′ to 5′ (see above), only one of the strands (known as the **leading** strand; violet/pink) can undergo *continuous* replication. For the **lagging strand** (light blue), the reading direction is the *opposite* of the direction of movement of the fork. In this matrix, the new strand is first synthesized in individual pieces, which are known as **Okazaki fragments** after their discoverer (green/orange).

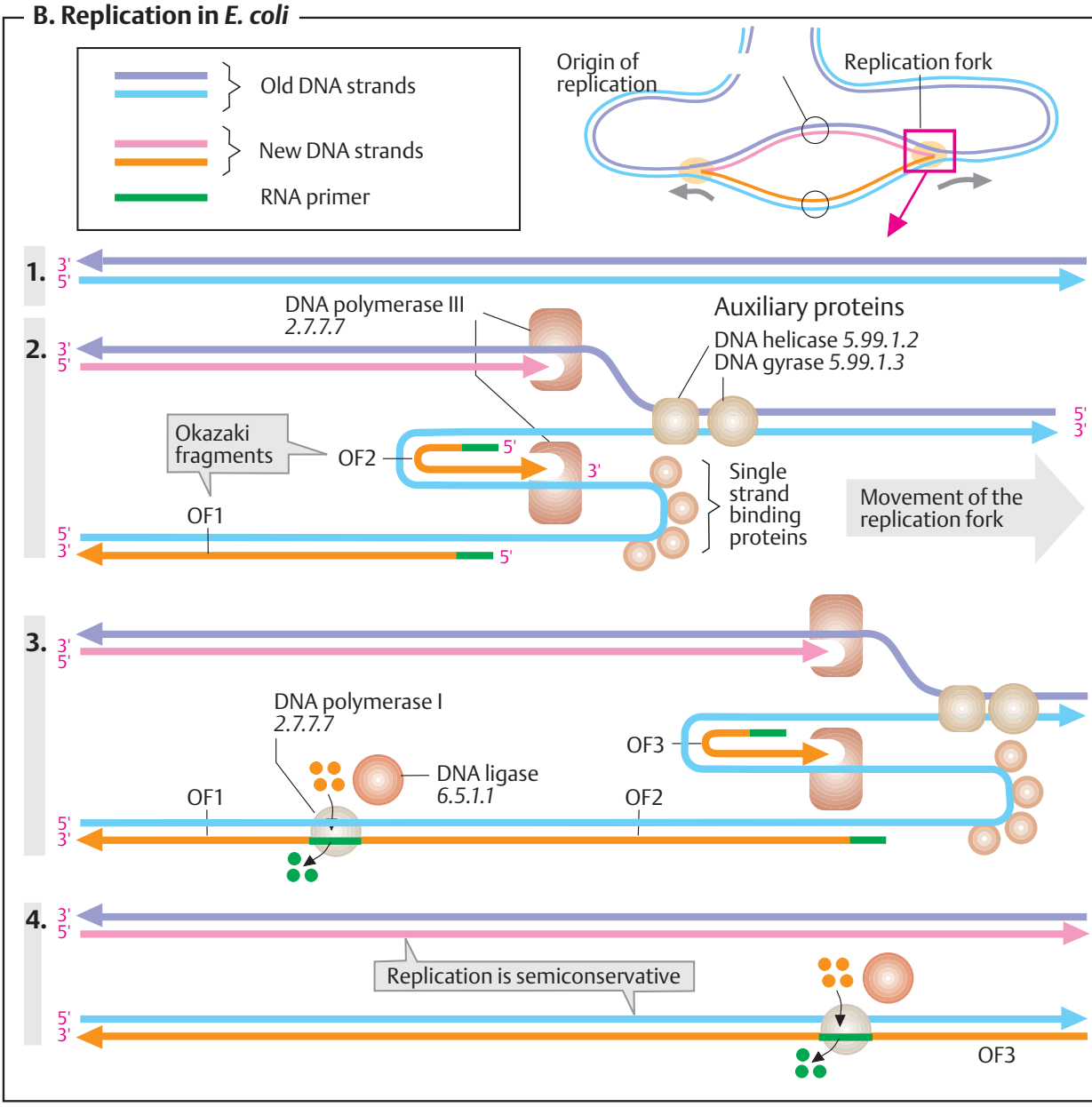
Each fragment starts with a short RNA primer (green), which is necessary for the functioning of the DNA polymerase and is synthesized by a special *RNA polymerase* (*"primase,"* not shown). The primer is then extended by *DNA polymerase III* (orange). After 1000–2000 nucleotides have been included, synthesis of the fragment is interrupted and a new one is begun, starting with another RNA primer that has been synthesized in the interim. The individual Okazaki fragments are initially not bound to one another and still have RNA at the 5′ end (**3**). At some distance from the fork, *DNA polymerase I* therefore starts to remove the RNA primer and replace it with DNA components. Finally, the gaps still remaining are closed by a *DNA ligase.* In DNA double helices formed in this way, only *one* of the strands has been newly synthesized—i. e., replication is *semiconservative*.

In bacteria, some 1000 nucleotides are replicated per second. In eukaryotes, replication takes place more slowly (about 50 nucleotides s^{-1}) and the genome is larger. Thousands of replication forks are therefore active simultaneously in eukaryotes.

A. Mechanism of DNA polymerases



B. Replication in *E. coli*



Transcription

For the genetic information stored in DNA to become effective, it has to be rewritten (transcribed) into RNA. DNA only serves as a template and is not altered in any way by the **transcription** process. Transcribable segments of DNA that code for a defined product are called **genes**. It is estimated that the mammalian genome contains 30 000–40 000 genes, which together account for less than 5% of the DNA.

A. Transcription and maturation of RNA: overview ◑

Transcription is catalyzed by *DNA–dependent RNA polymerases.* These act in a similar way to DNA polymerases (see p. 240), except that they incorporate *ribonucleotides* instead of deoxyribonucleotides into the newly synthesized strand; also, they do not require a primer. Eukaryotic cells contain at least three different types of RNA polymerase. *RNA polymerase I* synthesizes an RNA with a sedimentation coef cient (see p. 200) of 45 S, which serves as precursor for three ribosomal RNAs. The products of *RNA polymerase II* are hnRNAs, from which mRNAs later develop, as well as precursors for snRNAs. Finally, *RNA polymerase III* transcribes genes that code for tRNAs, 5S rRNA, and certain snRNAs. These precursors give rise to functional RNA molecules by a process called **RNA maturation** (see p. 246). Polymerases II and III are inhibited by *α–amanitin,* a toxin in the *Amanita phalloides* mushroom.

B. Organization of the PEP-CK gene ○

The way in which a typical eukaryotic gene is organized is illustrated here using a gene that codes for a key enzyme in gluconeogenesis (see p. 154)—the *phosphoenolpyruvate carboxykinase* (PEP-CK).

In the rat, the PEP-CK gene is nearly 7 kbp (kilobase pairs) long. Only 1863 bp, distributed over 10 coding segments (**exons**, dark blue) carry the information for the protein's 621 amino acids. The remainder is allotted to the promoter (pink) and intervening sequences (**introns**, light blue). The gene's promoter region (approximately 1 kbp) serves for regulation (see p. 188). Transcription starts at the 3′ end of the promoter ("transcription start") and continues until the polyadenylation sequence (see below) is reached. The primary transcript (**hnRNA**) still has a length of about 6.2 kbp. During RNA maturation, the noncoding sequences corresponding to the introns are removed, and the two ends of the hnRNA are modified. The translatable mRNA still has half the length of the hnRNA and is modified at both ends (see p. 246).

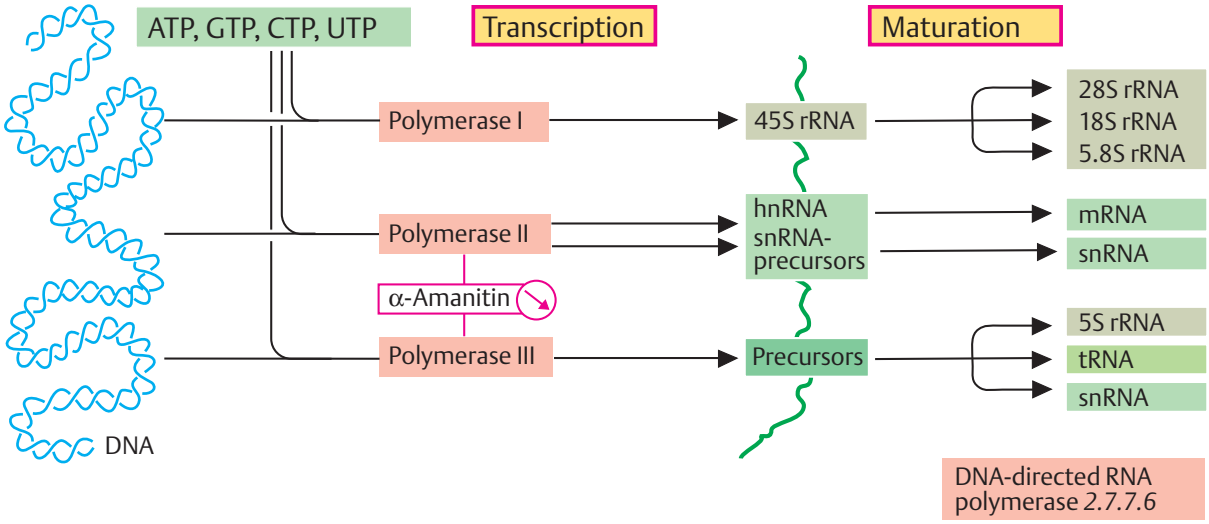
In many eukaryotic genes, the proportion of introns is even higher. For example, the gene for *dihydrofolate reductase* (see p. 402) is over 30 kbp long. The information is distributed over six exons, which together have a length of only about 6 kbp.

C. Transcription process ◑

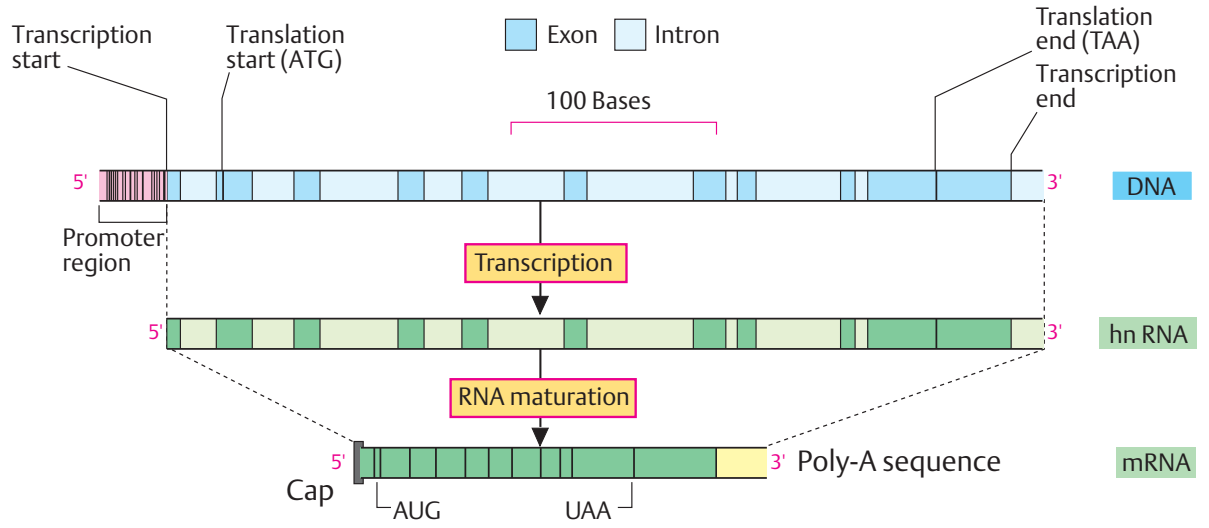
As mentioned above, *RNA polymerase II* (green) binds to the 3′ end of the promoter region. A sequence that is important for this binding is known as the **TATA box**—a short A– and T–rich sequence that varies slightly from gene to gene. A typical base sequence *("consensus sequence")* is …TATAAA… Numerous proteins known as *basal transcription factors* are necessary for the interaction of the polymerase with this region. Additional factors can promote or inhibit the process (transcriptional control; see p. 244). Together with the polymerase, they form the **basal transcription complex**.

At the end of **initiation** (**2**), the polymerase is repeatedly phosphorylated, frees itself from the basal complex, and starts moving along the DNA in the 3′ direction. The enzyme separates a short stretch of the DNA double helix into two single strands. The complementary nucleoside triphosphates are bound by base pairing in the *template strand* and are linked step by step to the hnRNA as it grows in the 5′→3′ direction (**3**). Shortly after the beginning of **elongation**, the 5′ end of the transcript is protected by a "cap" (see p. 246). Once the polyadenylation sequence has been reached (typical sequence: …AATAA…), the transcript is released (**4**). Shortly after this, the RNA polymerase stops transcribing and dissociates from the DNA.

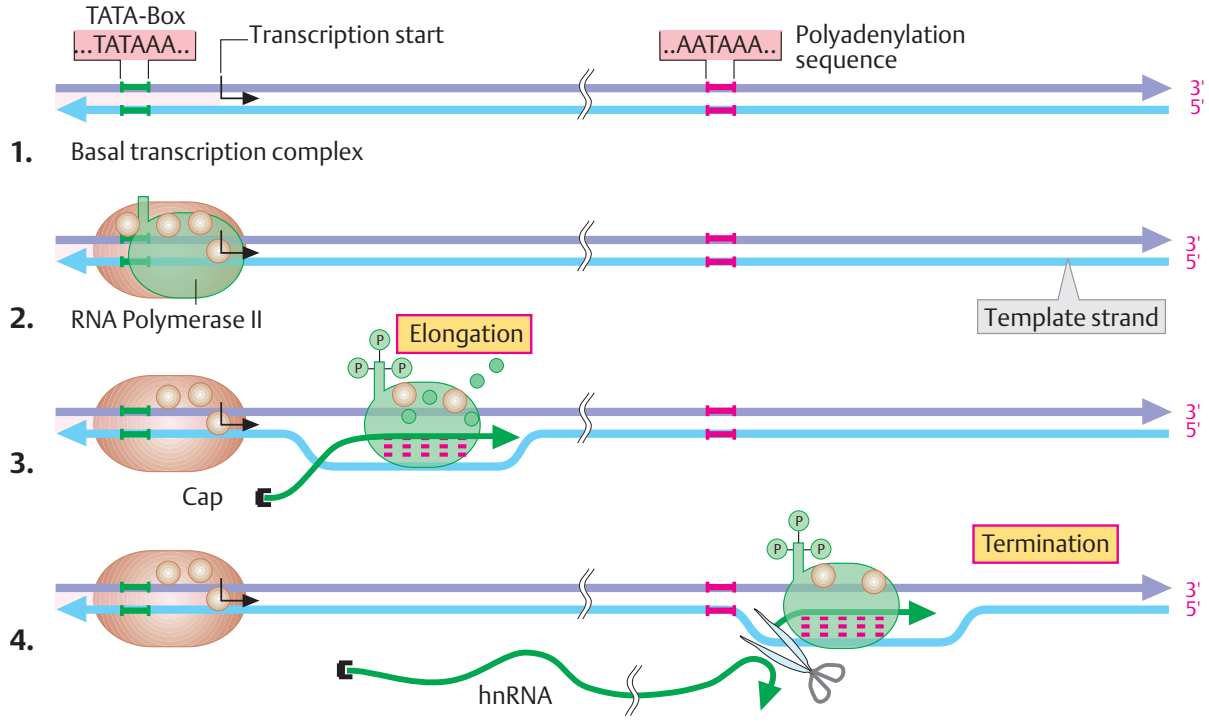
A. Transcription and maturation of RNA: overview



B. Organization of the PEP-CK gene



C. Process of transcription



Transcriptional control

Although all cells contain the complete genome, they only use a fraction of the information in it. The genes known as "housekeeping genes," which code for structural molecules and enzymes of intermediate metabolism, are the only ones that undergo constant transcription. The majority of genes are only active in certain cell types, in specific metabolic conditions, or during differentiation. Which genes are transcribed and which are not is regulated by **transcriptional control** (see also p. 118). This involves *control elements* (*cis*-active elements) in the gene's promoter region and gene-specific regulatory proteins (transcription factors, *trans* -active factors), which bind to the control elements and thereby activate or inhibit transcription.

A. Initiation of transcription ○

In the higher organisms, DNA is blocked by histones (see p. 238) and is therefore not capable of being transcribed without special positive regulation. In eukaryotes, it is therefore histones that play the role of *repressors* (see p. 118). For transcription to be set in motion at all, the chromatin first has to be restructured.

In the resting state, the lysine residues in the N-terminal "tail" of the histones (see p. 238) are not acetylated. In this state, which can be produced by *histone deacetylases* [1], the nucleosomes are stable. It is only the interaction of activator and regulator proteins with their control elements that allows the binding of coactivator complexes that have *histone acetylase* activity [2]. They acetylate the histone tails and thereby loosen the nucleosome structure suf ciently for the basal transcription complex to form.

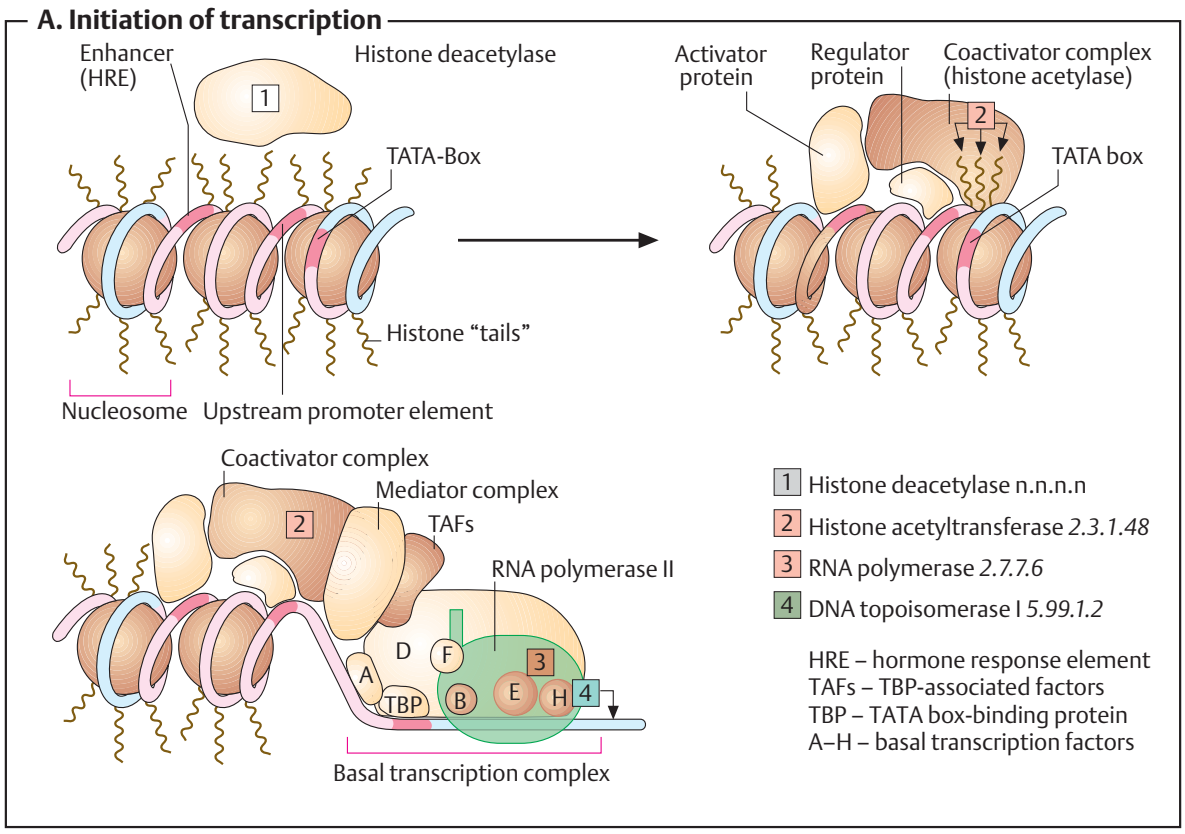
This consists of *DNA-dependent RNA polymerase II* and basal transcription factors (TFIIX, X = A – H). First, the basal factor TFIID binds to the promoter. TFIID, a large complex of numerous proteins, contains *TATA box-binding protein* (TBP) and so-called TAFs (TBP-associated factors). The polymerase is attached to this core with the help of TFIIB. Before transcription starts, additional TFs have to bind, including TFIIH, which has *helicase* activity and separates the two strands of DNA during elongation. In all, some 35 different proteins are involved in the basal complex. This alone, however, is still not suf cient for transcription to start. In addition, positive signals have to be emitted by more distant *trans*-active factors, integrated by the coactivator/mediator complex, and passed on to the basal complex (see **B**).

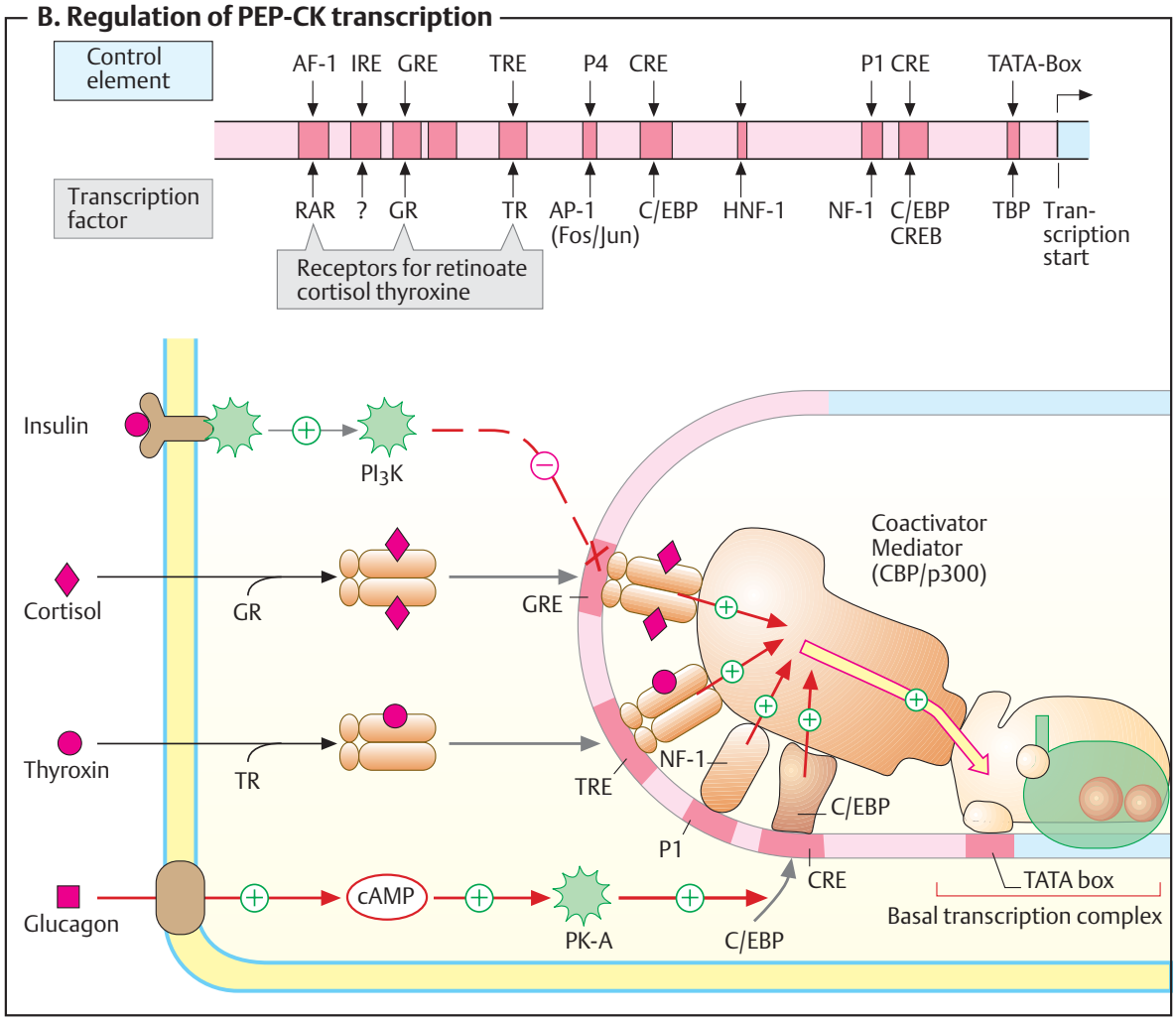
The actual signal for starting elongation consists of the multiple phosphorylation of a domain in the C-terminal region of the polymerase. In phosphorylated form, it releases itself from the basal complex along with a few TFs and starts to synthesize hnRNA.

B. Regulation of PEP-CK transcription ○

Phosphoenolpyruvate carboxykinase (PEP-CK), a key enzyme in gluconeogenesis, is regulated by several hormones, all of which affect the transcription of the PEP-CK gene. Cortisol, glucagon, and thyroxin induce PEP-CK, while insulin inhibits its induction (see p. 158).

More than ten **control elements** (dark red), distributed over approximately 1 kbp, have so far been identified in the promoter of the PEP-CK gene (top). These include response elements for the glucocorticoid receptor (GRE; see p. 378), for the thyroxin receptor (TRE), and for the steroid-like retinoic acid (AF-1). Additional control elements (CRE, cAMP-responsive element) bind the transcription factor C/EBP, which is activated by cAMP-dependent protein kinase A through phosphorylation. This is the way in which glucagon, which raises the cAMP level (see p. 158), works. Control element P1 binds the hormone-independent factor NF-1 (nuclear factor-1). All proteins that bind to the control elements mentioned above are in contact with a **coactivator/mediator complex** (CBP/p300), which integrates their input like a computer and transmits the result in the form of stronger or weaker signals to the basal transcription complex. Inhibition of PEP-CK transcription by insulin is mediated by an insulin-responsive element (IRE) in the vicinity of the GRE. Binding of an as yet unknown factor takes place here, inhibiting the binding of the glucocorticoid receptor to the GRE.





RNA maturation

Before the hnRNA produced by RNA polymerase II (see p. 242) can leave the nucleus in order to serve as a template for protein synthesis in the cytoplasm, it has to undergo several modifications first. Even during transcription, the two ends of the transcript have additional nucleotides added (**A**). The sections that correspond to the intervening gene sequences in the DNA (introns) are then cut out (splicing; see **B**). Other transcripts—e.g., the 45 S precursor of rRNA formed by polymerase I (see p. 242)—are broken down into smaller fragments by nucleases before export into the cytoplasm.

A. 5′ and 3′ modification of mRNA ◑

Shortly after transcription begins in eukaryotes, the end of the growing RNA is blocked in several reaction steps by a structure known as a "**cap**." In hnRNAs, this consists of a GTP residue that is methylated at N-7 of the guanine ring. The β-phosphate residue of the cap is linked to the free 5′-OH group of the terminal ribose via an ester bond. After the "polyadenylation signal" has been reached (typical sequence: …AAUAAA…; see p. 242), a **polyadenylate "tail"** consisting of up to 200 AMP nucleotides is also added at the free 3′ end of the transcript. This reaction is catalyzed by a special *polyadenylate polymerase*. It is only at this point that the mRNA leaves the nucleus as a complex with RNA-binding proteins.

Both the cap and the poly-A tail play a vital part in initiating eukaryotic translation (see p. 250). They help position the ribosome correctly on the mRNA near to the starting codon. The protection which the additional nucleotides provide against premature enzymatic degradation appears to be of lesser importance.

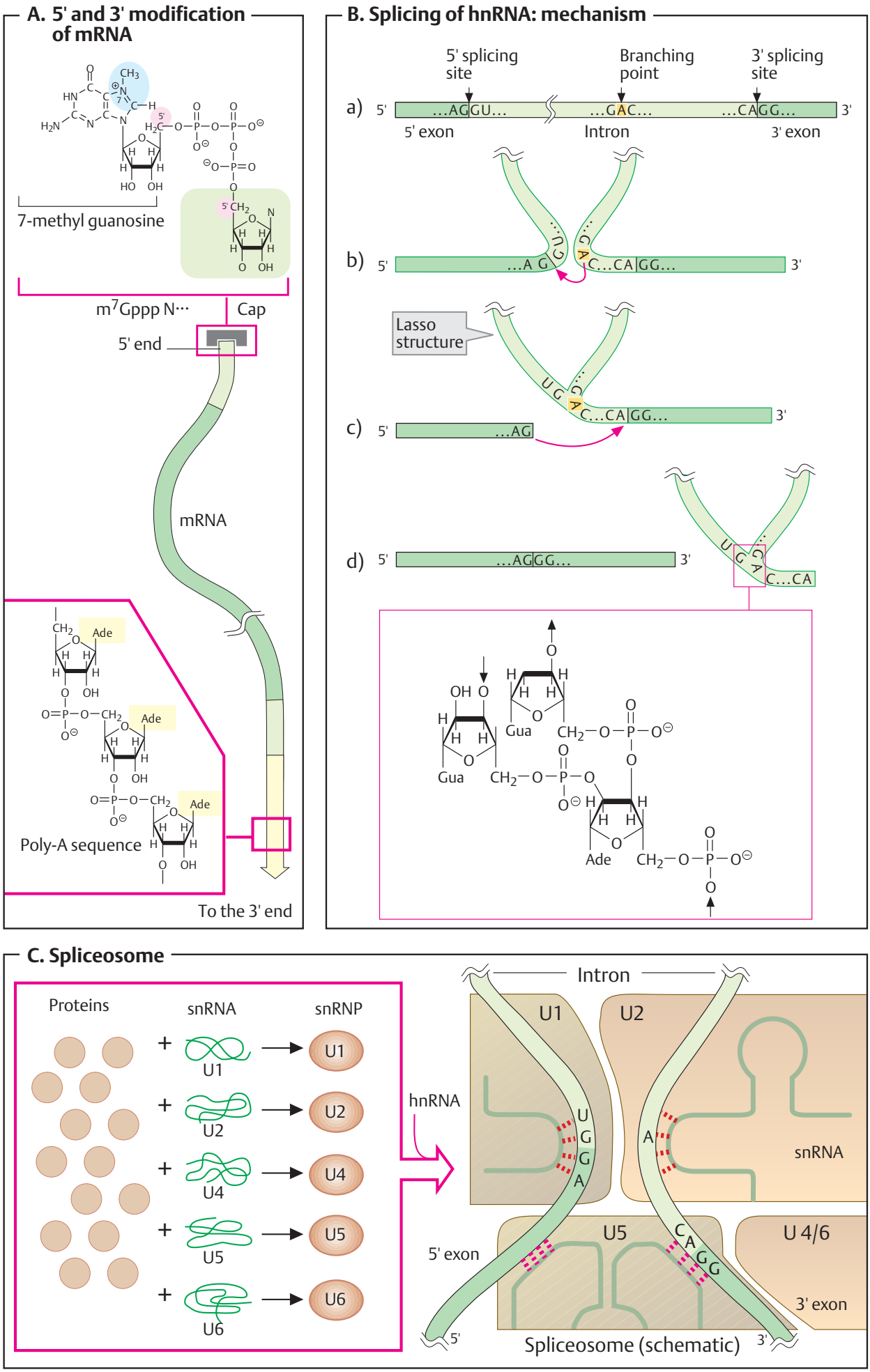
B. Splicing of hnRNA ◑

Immediately after transcription, the hnRNA introns are removed and the exons are linked to form a continuous coding sequence. This process, known as **splicing**, is supported by complicated RNA–protein complexes in the nucleus, the so-called **spliceosomes**. The components of these macromolecular machines are called **snRNPs** (*small nuclear ribonucleoprotein particles,* pronounced "snurps"). SnRNPs occur in five different forms (U1, U2, U4, U5, and U6). They consist of numerous *proteins* and one molecule of *snRNA* each (see p. 82).

To ensure that the RNA message is not destroyed, splicing has to take place in a very precise fashion. The start and end of the hnRNA introns are recognized by a characteristic sequence (…AGGT… at the 5′ end or …[C,U]AGG… at the 3′ end). Another important structure is the so-called *branching point* inside the intron. Its sequence is less conserved than the terminal splicing sites, but it always contains one adenosine residue (A). During splicing, the 2′-OH group of this residue—supported by the spliceosome (see **C**)—attacks the phosphoric acid diester bond at the 5′ end of the intron and cleaves it (**b**). Simultaneously, an unusual 2′→5′ bond is formed inside the intron, which thereby takes on a *lasso shape* (**c**; see formula). In the second step of the splicing process, the free 3′-OH group at the end of the 5′ terminal exon attacks the A–G bond at the 3′ end of the intron. As a result, the two exons are linked and the intron is released, still in a lasso shape (d).

C. Spliceosome ○

As described above, it is residues of the hnRNA that carry out bond cleavage and bond formation during the splicing process. It is therefore not a protein enzyme that acts as a catalyst here, but rather an RNA. Catalytic RNAs of this type are called *ribozymes* (see also p. 88). The task of the spliceosomes is to fix and orientate the reacting groups by establishing base pairings between snRNAs and segments of the hnRNA. The probable situation before the adenosine attack at the branching point on the 5′ splicing site (see **B**, Fig. **b**) is shown schematically on the right side of the illustration. In this phase, the U1 snRNA fixes the 5′ splicing site, U2 fixes the branching site, and U5 fixes the ends of the two exons.



Amino acid activation

A. The genetic code ◑

Most of the genetic information stored in the genome codes for the amino acid sequences of proteins. For these proteins to be expressed, a text in "nucleic acid language" therefore has to be translated into "protein language." This is the origin of the use of the term **translation** to describe protein biosynthesis. The dictionary used for the translation is the **genetic code.**

As there are 20 proteinogenic amino acids (see p. 60), the nucleic acid language has to contain at least as many words **(codons)**. However, there are only four letters in the nucleic acid alphabet (A, G, C, and U or T). To obtain 20 different words from these, each word has to be at least three letters long (with two letters, there would only be $4^2 = 16$ possibilities). And in fact the codons do consist of three sequential bases **(triplets)**.

Figure 1 shows the standard code in "DNA language" (i. e., as a sequence of triplets in the *sense strand* of DNA, read in the 5′→3′ direction; see p. 84), represented as a circular diagram. The scheme is read from the inside to the outside. For example, the triplet CAT codes for the amino acid histidine. With the exception of the exchange of U for T, the DNA codons are identical to those of mRNA.

As the genetic code provides $4^3 = 64$ codons for the 20 amino acids, there are several synonymous codons for most amino acids—the code is **degenerate**. Three triplets do not code for amino acids, but instead signal the end of translation **(stop codons)**. Another special codon, the **start codon**, marks the start of translation. The code shown here is almost universally applicable; only the mitochondria (see p. 210) and a few microorganisms deviate from it slightly.

As an example of the way in which the code is read, Fig. 2 shows small sections from the normal and a mutated form of the β-globin gene (see p. 280), as well as the corresponding mRNA and protein sequences. The **point mutation** shown, which is relatively frequent, leads to replacement of a glutamate residue in position 6 of the β-chain by valine (G**A**G → G**T**G). As a consequence, the mutated hemoglobin tends to aggregate in the deoxygenated form. This leads to sickle-shaped distortions of the erythrocytes and disturbances of O_2 transport (sickle-cell anemia).

B. Amino acid activation ◑

Some 20 different *amino acid tRNA ligases* in the cytoplasm each bind one type of tRNA (see p. 82) with the corresponding amino acid. This reaction, known as **amino acid activation**, is endergonic and is therefore coupled to ATP cleavage in two steps.

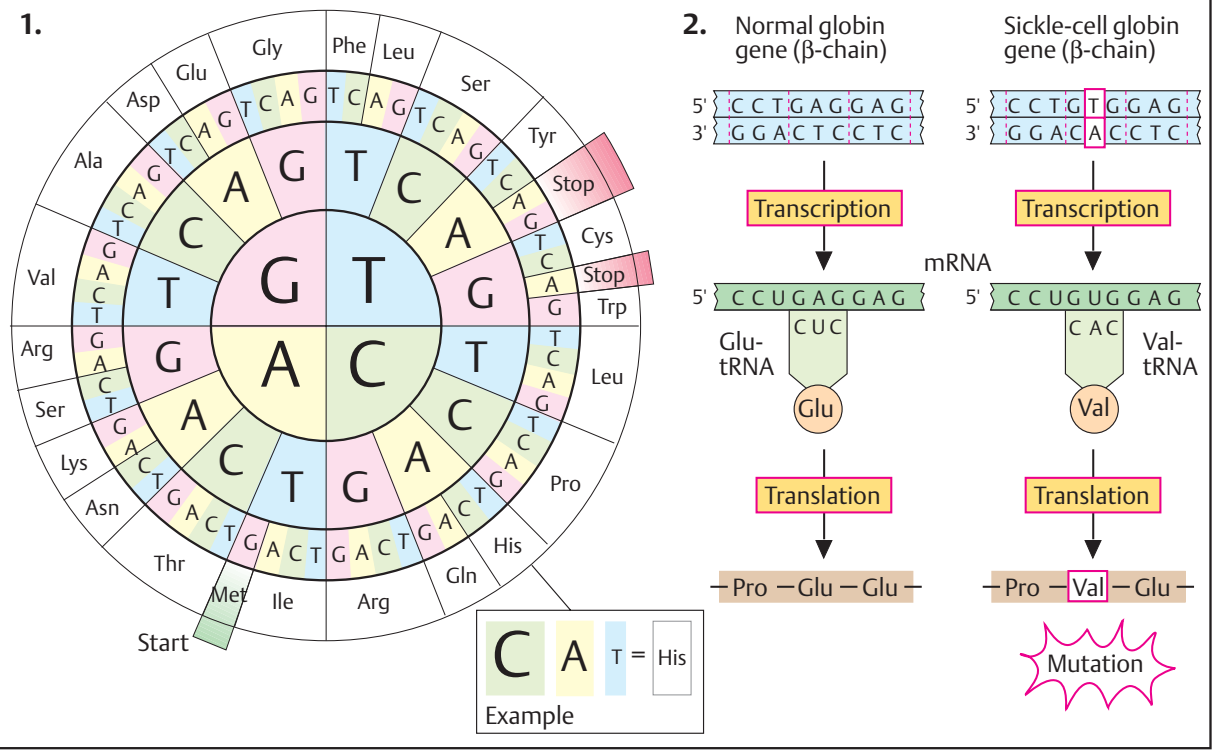
First, the amino acid is bound by the enzyme and reacts there with ATP to form diphosphate and an "energy–rich" mixed acid anhydride (**aminoacyl adenylate**). In the second step, the 3′-OH group (in other ligases it is the 2′-OH group) of the terminal ribose residue of the tRNA takes over the amino acid residue from the aminoacyl adenylate. In **aminoacyl tRNAs**, the carboxyl group of the amino acid is therefore esterified with the ribose residue of the terminal adenosine of the sequence …CCA-3′.

The accuracy of translation primarily depends on the specificity of the amino acid tRNA ligases, as incorrectly incorporated amino acid residues are not recognized by the ribosome later. A *"proofreading mechanism"* in the active center of the ligase therefore ensures that incorrectly incorporated amino acid residues are immediately removed again. On average, an error only occurs once every 1300 amino acid residues. This is a surprisingly low rate considering how similar some amino acids are—e. g., leucine and isoleucine.

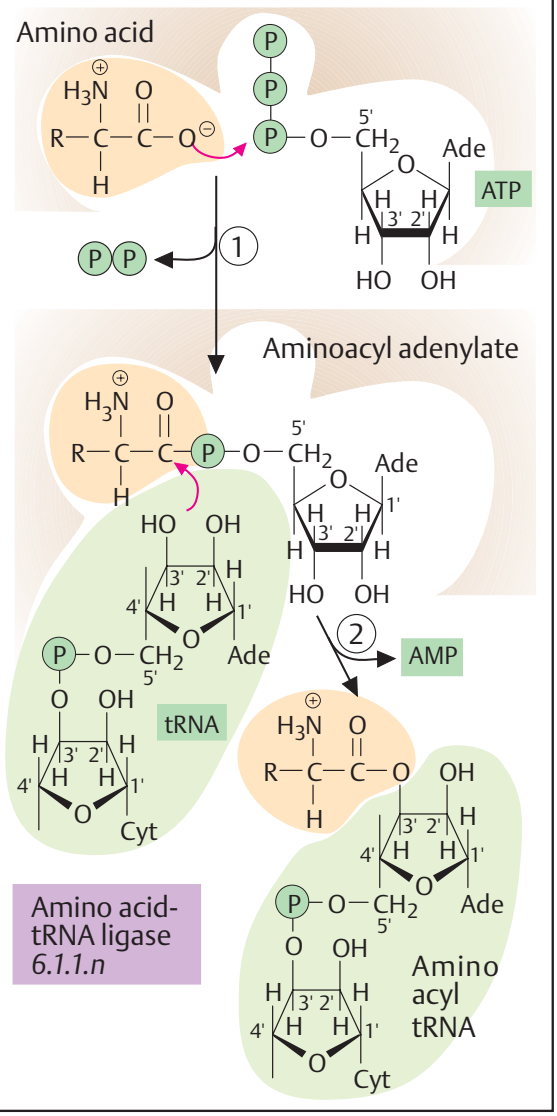
C. Asp–tRNA ligase (dimer) ○

The illustration shows the ligase responsible for the activation of aspartate. Each subunit of the dimeric enzyme (protein parts shown in orange) binds one molecule of $tRNA^{Asp}$ (blue). The active centers can be located by the bound ATP (green). They are associated with the 3′ end of the tRNA. Another domain in the protein (upper left) is responsible for "recognition" of the tRNA anticodon.

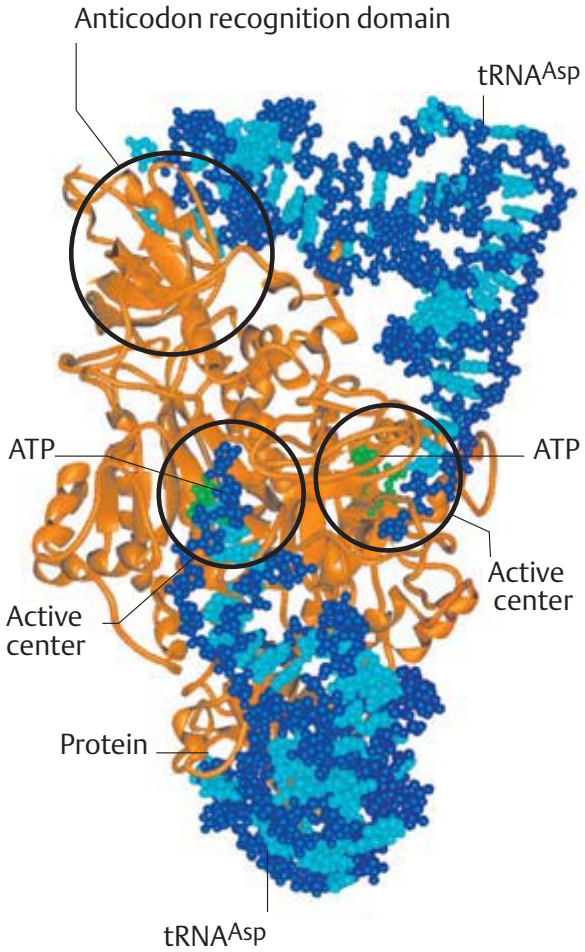
A. The genetic code



B. Amino acid activation



C. Asp-tRNA-Ligase (Dimer)



Translation I: initiation

Like amino acid activation (see p. 248), protein biosynthesis (**translation**) takes place in the cytoplasm. It is catalyzed by complex nucleoprotein particles, the **ribosomes**, and mainly requires GTP to cover its energy requirements.

A. Structure of the eukaryotic ribosome ◑

Ribosomes consist of two subunits of different size, made up of **ribosomal RNA (rRNA)** and nearly 80 **proteins** (the number of proteins applies to rat liver ribosomes). It is customary to give the sedimentation coef cients (see p. 200) of ribosomes and their components instead of their masses. For example, the eukaryotic ribosome has a sedimentation coefficient of 80 Svedberg units (80 S), while the sedimentation coef cients of its subunits are 40 S and 60 S (S values are not additive).

The smaller 40 S subunit consists of one molecule of 18 S rRNA and 33 protein molecules. The larger 60 S subunit contains three types of rRNA with sedimentation coef cients of 5 S, 5.8 S, and 28 S and 47 proteins. In the presence of mRNA, the subunits assemble to form the complete ribosome, with a mass about 650 times larger than that of a hemoglobin molecule.

The arrangement of the individual components of a ribosome has now been determined for prokaryotic ribosomes. It is known that filamentous mRNA passes through a cleft between the two subunits near the characteristic "horn" on the small subunit. tRNAs also bind near this site. The illustration shows the size of a tRNA molecule for comparison.

Prokaryotic ribosomes have a similar structure, but are somewhat smaller than those of eukaryotes (sedimentation coef cient 70 S for the complete ribosome, 30 S and 50 S for the subunits). Mitochondrial and chloroplast ribosomes are comparable to prokaryotic ones.

B. Polysomes ◑

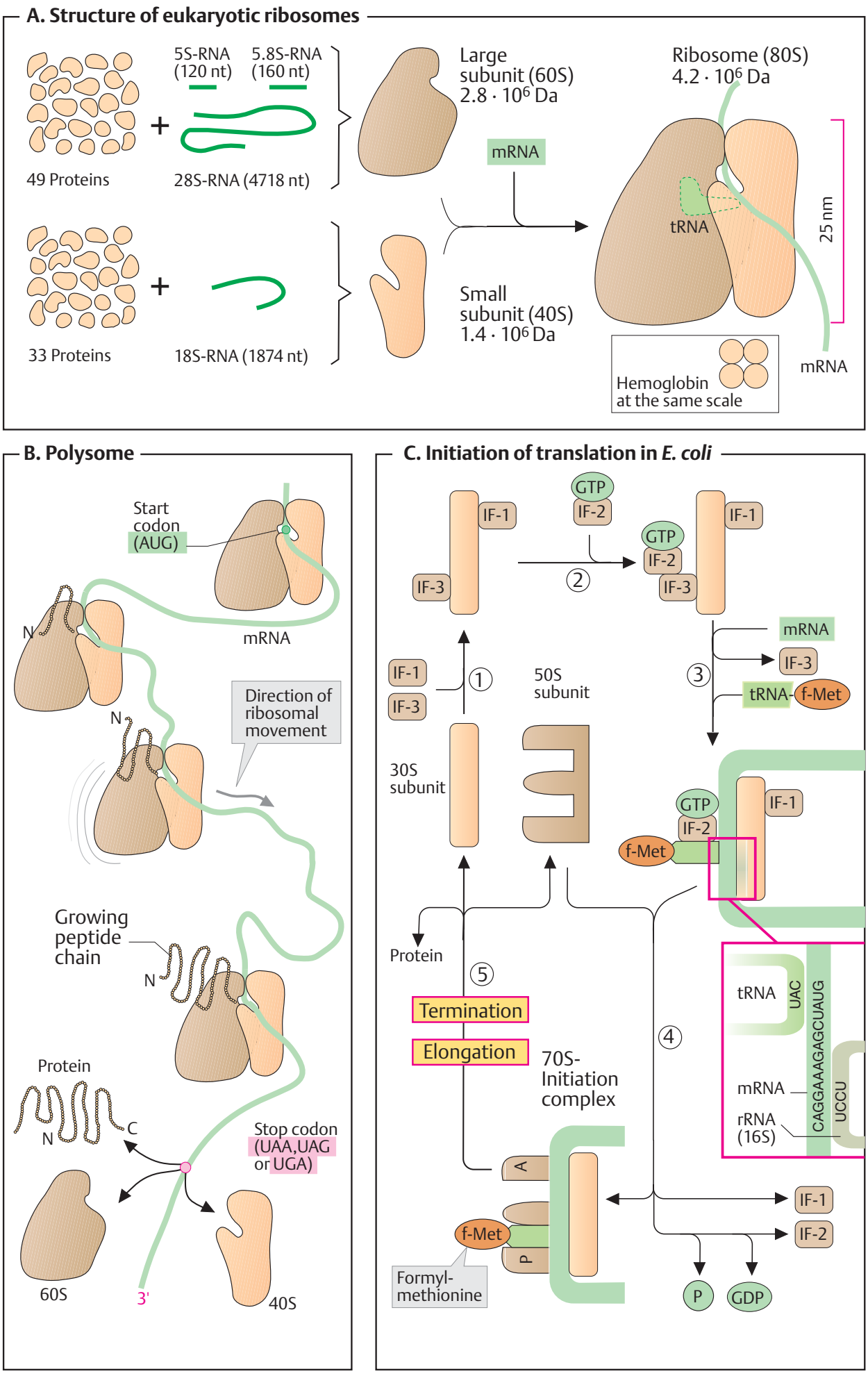
In cells that are carrying out intensive protein synthesis, ribosomes are often found in a linear arrangement like a string of pearls; these are known as **polysomes**. This arrangement arises because several ribosomes are translating a single mRNA molecule simultaneously. The ribosome first binds near the **start codon** (AUG; see p. 248) at the 5′ end of the mRNA (top). During translation, the ribosome moves in the direction of the 3′ end until it reaches a **stop codon** (UAA, UAG, or UGA). At this point, the newly synthesized chain is released, and the ribosome dissociates again into its two subunits.

C. Initiation of translation in E. coli ◑

Protein synthesis in prokaryotes is in principle the same as in eukaryotes. However, as the process is simpler and has been better studied in prokaryotes, the details involved in translation are discussed here and on p. 252 using the example of the bacterium *Escherichia coli.*

The first phase of translation, **initiation**, involves several steps. First, two proteins, **initiation factors** IF–1 and IF–3, bind to the 30 S subunit (**1**). Another factor, IF–2, binds as a complex with GTP (**2**). This allows the subunit to associate with the mRNA and makes it possible for a special tRNA to bind to the start codon (**3**). In prokaryotes, this starter tRNA carries the substituted amino acid *N-formylmethionine* (fMet). In eukaryotes, it carries an unsubstituted *methionine*. Finally, the 50 S subunit binds to the above complex (**4**). During steps 3 and 4, the initiation factors are released again, and the GTP bound to IF–2 is hydrolyzed to GDP and P_i.

In the **70 S initiation complex**, formylmethionine tRNA is initially located at a binding site known as the **peptidyl site (P)**. A second binding site, the **acceptor site (A)**, is not yet occupied during this phase of translation. Sometimes, a third tRNA binding site is defined as an *exit site (E),* from which uncharged tRNAs leave the ribosome again (see p. 252; not shown).



Translation II: elongation and termination

After translation has been initiated (see p. 250), the peptide chain is extended by the addition of further amino acid residues (**elongation**) until the ribosome reaches a stop codon on the mRNA and the process is interrupted (**termination**).

A. Elongation and termination of protein biosynthesis in E. coli ◑

Elongation can be divided into four phases:

[1] **Binding of aminoacyl tRNA.** First, the peptidyl site (P) of the ribosome is occupied by a tRNA that carries at its 3′ end the complete peptide chain formed up to this point (top left). A second tRNA, loaded with the next amino acid (Val–$tRNA^{Val}$ in the example shown), then binds via its complementary anticodon (see p. 82) to the mRNA codon exposed at the acceptor site (in this case GUG). The tRNA binds as a complex with a GTP-containing protein, the *elongation factor Tu* (EF–Tu) (**1a**). It is only after the bound GTP has been hydrolyzed to GDP and phosphate that EF–Tu dissociates again (**1b**). As the binding of the tRNA to the mRNA is still loose before this, GTP hydrolysis acts as a delaying factor, making it possible to check whether the correct tRNA has been bound. A further protein, the *elongation factor Ts* (EF-Ts), later catalyzes the exchange of GDP for GTP and in this way regenerates the EF–Tu GTP complex. EF-Tu is related to the G proteins involved in signal transduction (see p. 384).

[2] **Synthesis of the peptide bond** takes place in the next step. Ribosomal *peptidyltransferase* catalyzes (without consumption of ATP or GTP) the transfer of the peptide chain from the tRNA at the P site to the NH_2 group of the amino acid residue of the tRNA at the A site. The ribosome's peptidyltransferase activity is not located in one of the ribosomal proteins, but in the 28 S rRNA. Catalytically active RNAs of this type are known as *ribozymes* (cf. p. 246). It is thought that the few surviving ribozymes are remnants of the *"RNA world"*—an early phase of evolution in which proteins were not as important as they are today.

[3] After the transfer of the growing peptide to the A site, the free tRNA at the P site dissociates and another GTP–containing elongation factor (EF-G GTP) binds to the ribosome. Hydrolysis of the GTP in this factor provides the energy for **translocation** of the ribosome. During this process, the ribosome moves three bases along the mRNA in the direction of the 3′ end. The tRNA carrying the peptide chain is stationary relative to the mRNA and reaches the ribosome's P site during translocation, while the next mRNA codon (in this case GUG) appears at the A site.

[4] The uncharged Val-tRNA then dissociates from the E site. The ribosome is now ready for the next elongation cycle.

When one of the three stop codons (UAA, UAG, or UGA) appears at the A site, **termination** starts.

[5] There are no complementary tRNAs for the stop codons. Instead, two *releasing factors* bind to the ribosome. One of these factors (RF–1) catalyzes hydrolytic cleavage of the ester bond between the tRNA and the C–terminus of the peptide chain, thereby releasing the protein.

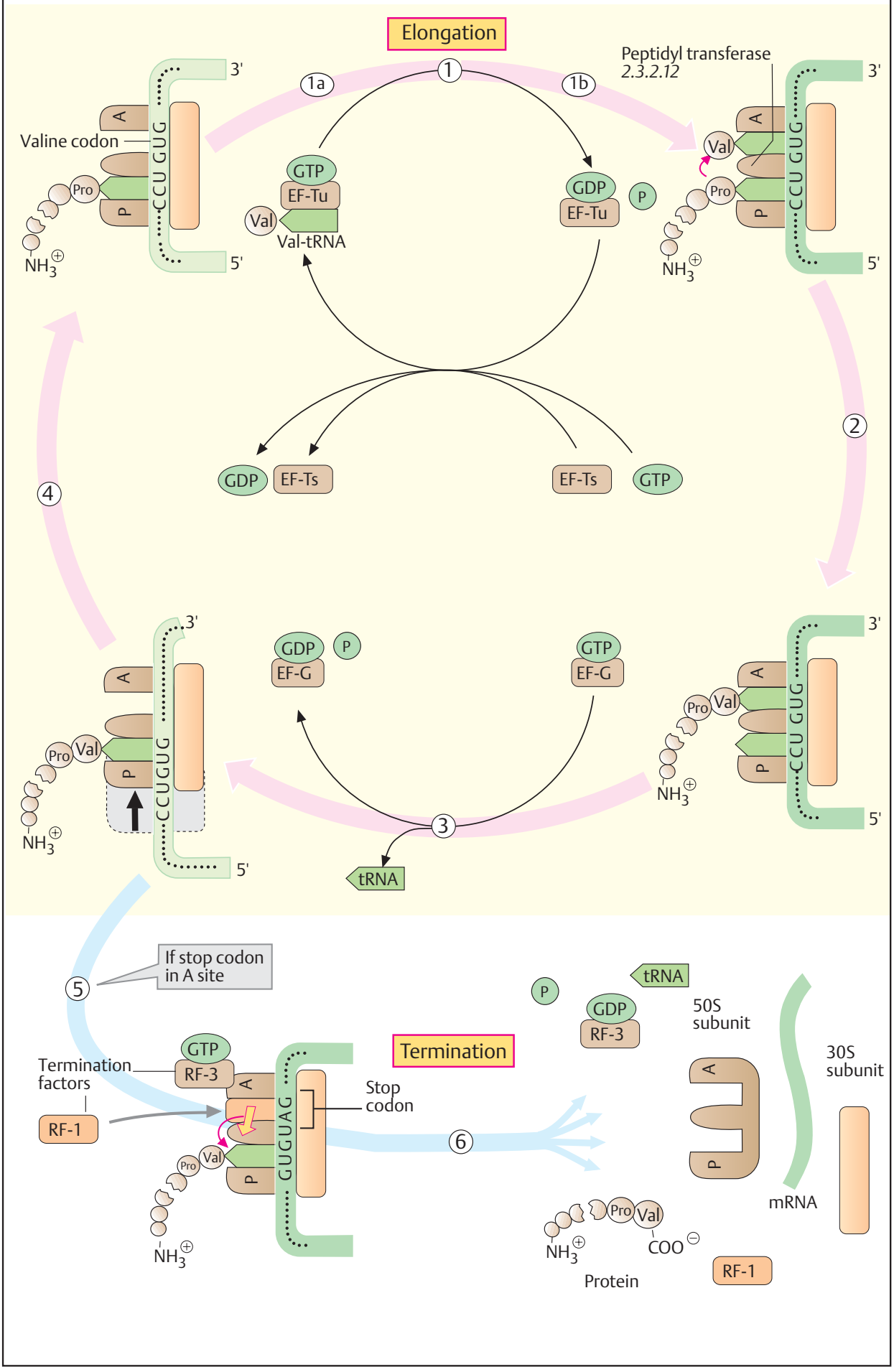
[6] Hydrolysis of GTP by factor RF–3 supplies the energy for the dissociation of the whole complex into its component parts.

Energy requirements in protein synthesis are high. Four energy-rich phosphoric acid anhydride bonds are hydrolyzed for each amino acid residue. Amino acid activation uses up two energy-rich bonds per amino acid (ATP → AMP + PP; see p. 248), and two GTPs are consumed per elongation cycle. In addition, initiation and termination each require one GTP per chain.

Further information

In eukaryotic cells, the number of initiation factors is larger and initiation is therefore more complex than in prokaryotes. The cap at the 5′ end of mRNA and the polyA tail (see p. 246) play important parts in initiation. However, the elongation and termination processes are similar in all organisms. The individual steps of bacterial translation can be inhibited by antibiotics (see p. 254).

A. Elongation and termination of protein biosynthesis in *E. coli*



Antibiotics

A. Antibiotics: overview ◑

Antibiotics are substances which, even at low concentrations, inhibit the growth and reproduction of bacteria and fungi. The treatment of infectious diseases would be inconceivable today without antibiotics. Substances that only restrict the reproduction of bacteria are described as having *bacteriostatic* effects (or *fungistatic* for fungi). If the target cells are killed, then the term *bactericidal* (or *fungicidal*) is used. Almost all antibiotics are produced by microorganisms—mainly bacteria of the genus *Streptomyces* and certain fungi. However, there are also synthetic antibacterial substances, such as sulfonamides and gyrase inhibitors.

A constantly increasing problem in antibiotic treatment is the development of resistant pathogens that no longer respond to the drugs available. The illustration shows a few of the therapeutically important antibiotics and their sites of action in the bacterial metabolism.

Substances known as **intercalators**, such as *rifamycin* and *actinomycin D* (bottom) are deposited in the DNA double helix and thereby interfere with replication and transcription (**B**). As DNA is the same in all cells, intercalating antibiotics are also toxic for eukaryotes, however. They are therefore only used as cytostatic agents (see p. 402). Synthetic inhibitors of DNA topoisomerase II (see p. 240), known as **gyrase inhibitors** (center), restrict replication and thus bacterial reproduction.

A large group of antibiotics attack bacterial ribosomes. These **inhibitors of translation** (left) include the *tetracyclines*—broad-spectrum antibiotics that are effective against a large number of different pathogens. The *aminoglycosides*, of which *streptomycin* is the best-known, affect all phases of translation. *Erythromycin* impairs the normal functioning of the large ribosomal subunit. *Chloramphenicol,* one of the few natural nitro compounds, inhibits ribosomal peptidyltransferase. Finally, *puromycin* mimics an aminoacyl tRNA and therefore leads to premature interruption of elongation.

The **β–lactam antibiotics** (bottom right) are also frequently used. The members of this group, the *penicillins* and *cephalosporins,* are synthesized by fungi and have a reactive β-lactam ring. They are mainly used against Gram-positive pathogens, in which they inhibit cell wall synthesis (**C**).

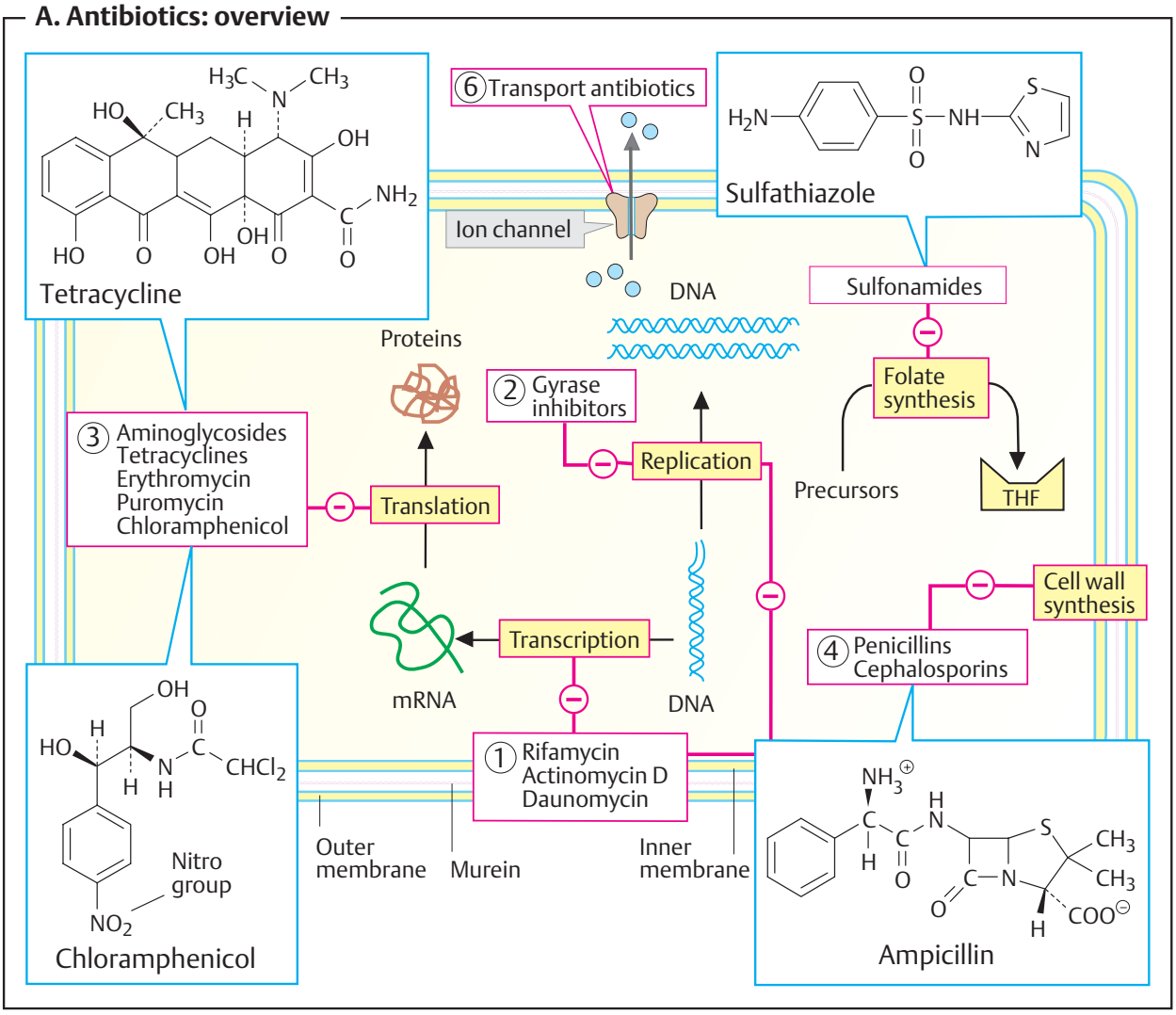
The first synthetic antibiotics were the **sulfonamides** (right). As analogues of p–aminobenzoic acid, these affect the synthesis of *folic acid,* an essential precursor of the coenzyme THF (see p. 108). **Transport antibiotics** (top center) have the properties of ion channels (see p. 222). When they are deposited in the plasma membrane, it leads to a loss of ions that damages the bacterial cells.

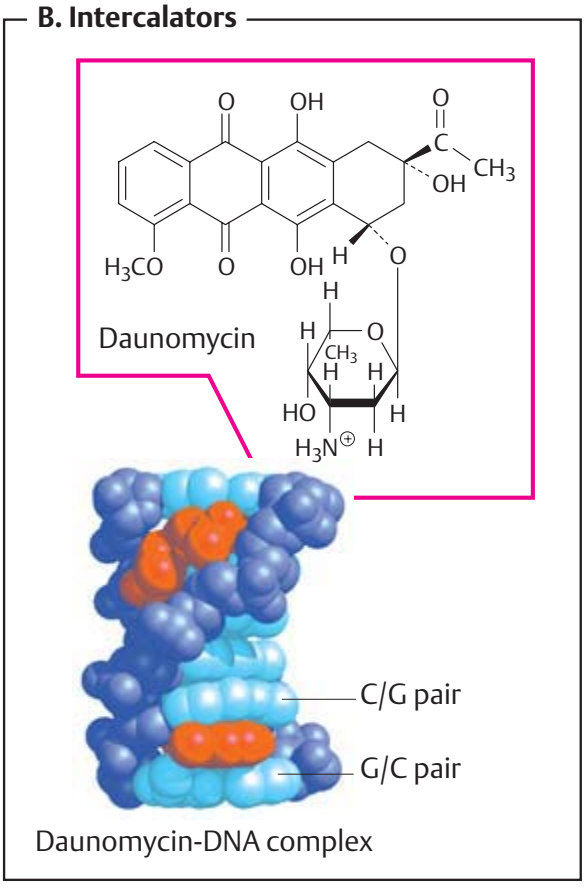
B. Intercalators ○

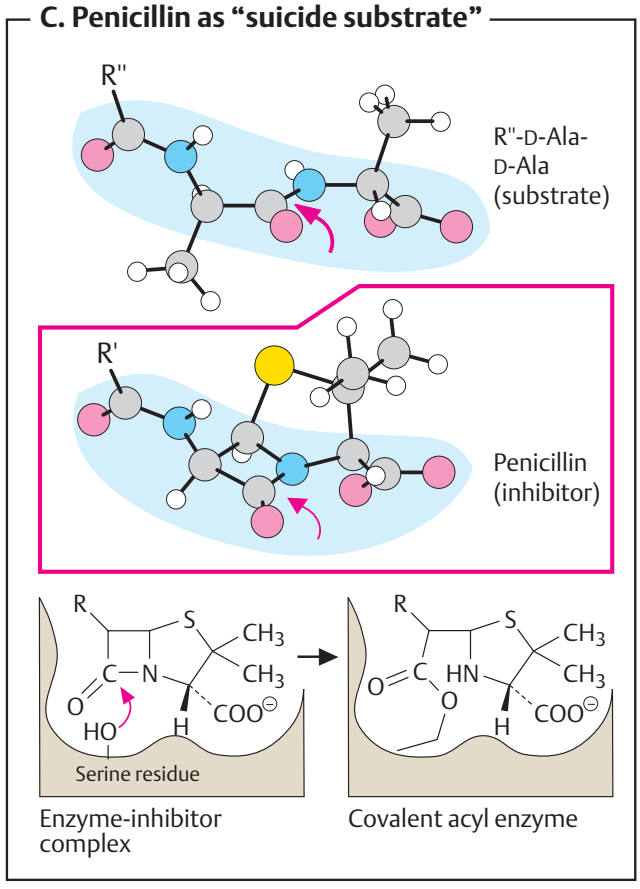
The effects of intercalators (see also p. 262) are illustrated here using the example of the **daunomycin–DNA complex**, in which two daunomycin molecules (red) are inserted in the double helix (blue). The antibiotic's ring system inserts itself between G/C base pairs (bottom), while the sugar moiety occupies the minor groove in the DNA (above). This leads to a localized change of the DNA conformation that prevents replication and transcription.

C. Penicillin as a "suicide substrate" ○

The site of action in the β–lactam antibiotics is *muramoylpentapeptide carboxypeptidase,* an enzyme that is essential for cross–linking of bacterial cell walls. The antibiotic resembles the substrate of this enzyme (a peptide with the C-terminal sequence D-Ala–D–Ala) and is therefore reversibly bound in the active center. This brings the β–lactam ring into proximity with an essential serine residue of the enzyme. Nucleophilic substitution then results in the formation of a stable covalent bond between the enzyme and the inhibitor, blocking the active center (see p. 96). In dividing bacteria, the loss of activity of the enzyme leads to the formation of unstable cell walls and eventually death.







Mutation and repair

Genetic information is set down in the base sequence of DNA. Changes in the DNA bases or their sequence therefore have *mutagenic* effects. Mutagens often also damage growth regulation in cells, and they are then also *carcinogenic* (see p. 400). Gene alterations (**mutations**) are one of the decisive positive factors in biological evolution. On the other hand, an excessive mutation frequency would threaten the survival of individual organisms or entire species. For this reason, every cell has **repair mechanisms** that eliminate most of the DNA changes arising from mutations (**C**).

A. Mutagenic agents ◑

Mutations can arise as a result of physical or chemical effects, or they can be due to accidental errors in DNA replication and recombination.

The principal physical mutagen is **ionizing radiation** (α, β, and γ radiation, X-rays). In cells, it produces **free radicals** (molecules with unpaired electrons), which are extremely reactive and can damage DNA. Short-wavelength **ultraviolet light** (UV light) also has mutagenic effects, mainly in skin cells (sunburn). The most common chemical change due to UV exposure is the formation of **thymine dimers**, in which two neighboring thymine bases become covalently linked to one another (**2**). This results in errors when the DNA is read during replication and transcription.

Only a few examples of the group of **chemical mutagens** are shown here. *Nitrous acid* (HNO_2; salt: nitrite) and *hydroxylamine* (NH_2OH) both deaminate bases; they convert cytosine to uracil and adenine to inosine.

Alkylating compounds carry reactive groups that can form covalent bonds with DNA bases (see also p. 402). *Methylnitrosamines* (**3**) release the reactive methyl cation (CH_3^+), which methylates OH and NH_2 groups in DNA. The dangerous carcinogen *benzo*[a]*pyrene* is an aromatic hydrocarbon that is only converted into the active form in the organism (**4**; see p. 316). Multiple hydroxylation of one of the rings produces a reactive epoxide that can react with NH_2 groups in guanine residues, for example. Free radicals of benzo[*a*]pyrene also contribute to its toxicity.

B. Effects ◑

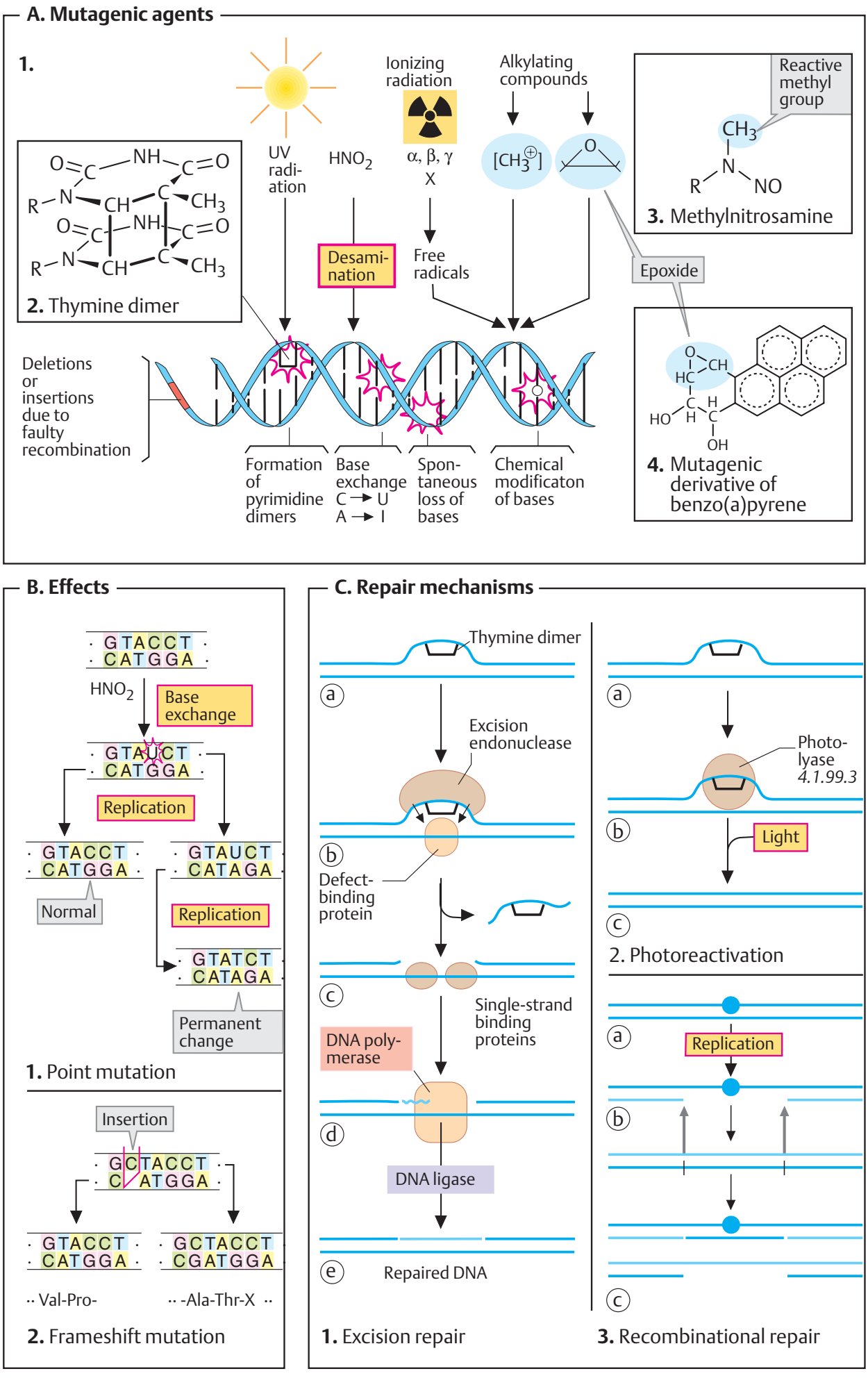
Nitrous acid causes **point mutations** (**1**). For example, C is converted to U, which in the next replication pairs with A instead of G. The alteration thus becomes permanent. Mutations in which a number of nucleotides not divisible by three are inserted or removed lead to reading errors in whole segments of DNA, as they move the reading frame (**frameshift mutations**). This is shown in Fig. **2** using a simple example. From the inserted C onwards, the resulting mRNA is interpreted differently during translation, producing a completely new protein sequence.

C. Repair mechanisms ○

An important mechanism for the removal of DNA damage is **excision repair** (**1**). In this process, a specific *excision endonuclease* removes a complete segment of DNA on both sides of the error site. Using the sequence of the opposite strand, the missing segment is then replaced by a *DNA polymerase*. Finally, a *DNA ligase* closes the gaps again.

Thymine dimers can be removed by **photoreactivation** (**2**). A specific *photolyase* binds at the defect and, when illuminated, cleaves the dimer to yield two single bases again.

A third mechanism is **recombination repair** (**3**, shown in simplified form). In this process, the defect is omitted during replication. The gap is closed by shifting the corresponding sequence from the correctly replicated second strand. The new gap that results is then filled by polymerases and ligases. Finally, the original defect is corrected by excision repair as in Fig. **1** (not shown).



DNA cloning

The growth of molecular genetics since 1970 has mainly been based on the development and refinement of methods of analyzing and manipulating DNA. **Genetic engineering** has practical applications in many fields. For example, it has provided new methods of diagnosing and treating diseases, and it is now also possible to create targeted changes in specific characteristics of organisms. Since biological risks cannot be completely ruled out with these procedures, it is particularly important to act responsibly when dealing with genetic engineering. A short overview of important methods involved in genetic engineering is provided here and on the following pages.

A. Restriction endonucleases ◑

In many genetic engineering procedures, defined DNA fragments have to be isolated and then newly combined with other DNA segments. For this purpose, enzymes are used that can cut DNA and join it together again inside the cell. Of particular importance are *restriction endonucleases*—a group of bacterial enzymes that cleave the DNA double strand in a sequence–specific way. The numerous restriction enzymes known are named using abbreviations based on the organism from which they originate. The example used here is *Eco*RI, a nuclease isolated from the bacterium *Escherichia coli.*

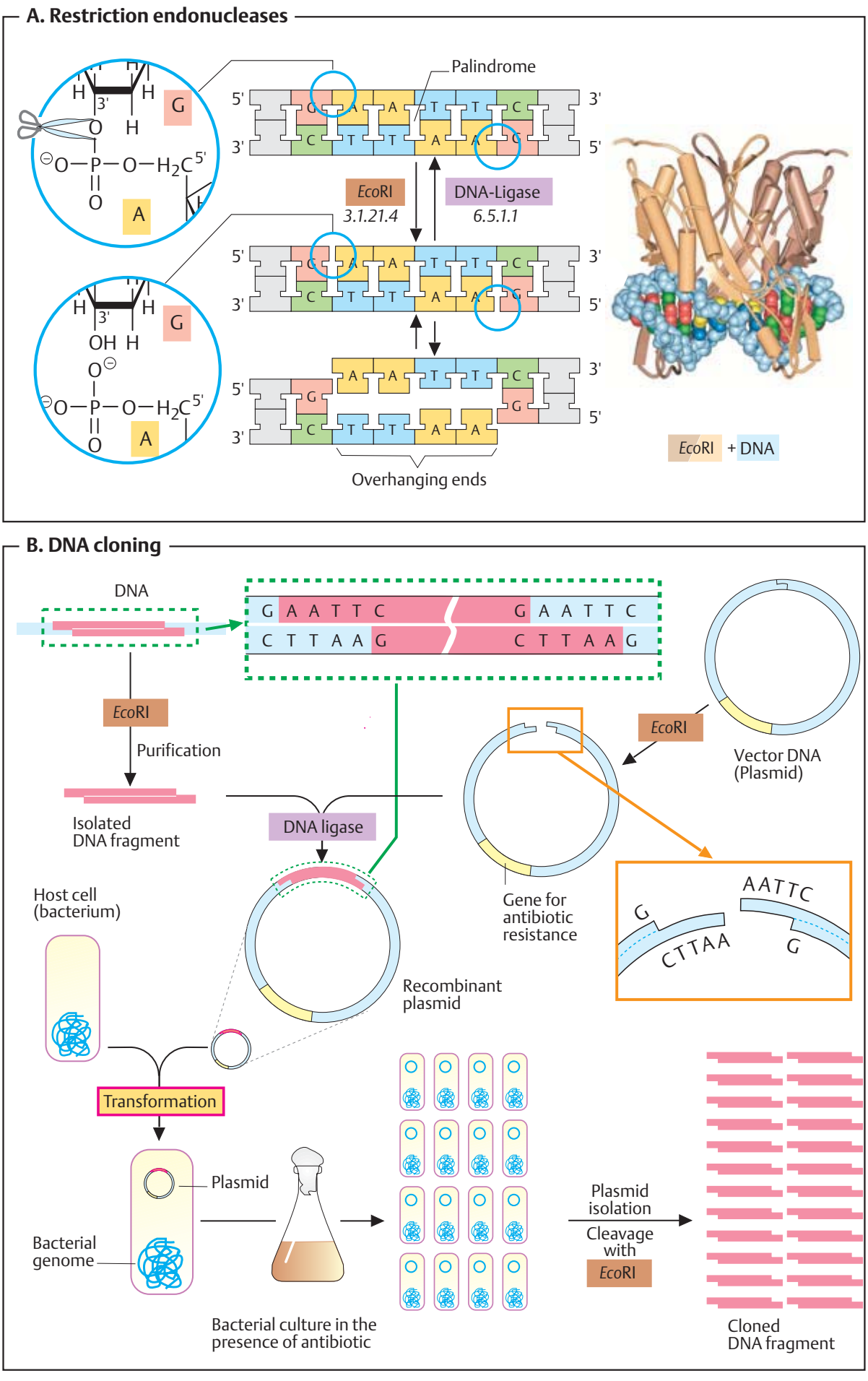
Like many other restriction endonucleases, EcoRI cleaves DNA at the site of a *palindrome*—i. e., a short segment of DNA in which both the strand and counter-strand have the same sequence (each read in the 5′→3′ direction). In this case, the sequence is 5′-GAATTC-3′. *Eco*RI, a homodimer, cleaves the phosphoric acid diester bonds in both strands between G and A. This results in the formation of complementary *overhanging* or *"sticky" ends* (AATT), which are held together by base pairing. However, they are easily separated—e. g. by heating. When the fragments are cooled, the overhanging ends hybridize again in the correct arrangement. The cleavage sites can then be sealed again by a *DNA ligase*.

B. DNA cloning ○

Most DNA segments—e. g., genes—occur in very small quantities in the cell. To be able to work with them experimentally, a large number of identical copies (**"clones"**) first have to be produced. The classic procedure for cloning DNA takes advantage of the ability of bacteria to take up and replicate short, circular DNA fragments known as **plasmids**.

The segment to be cloned is first cut out of the original DNA using restriction endonucleases (see above; for the sake of simplicity, cleavage using *Eco*RI alone is shown here, but in practice two different enzymes are usually used). As a vehicle (*"vector"*), a plasmid is needed that has only *one Eco*RI cleavage site. The plasmid rings are first opened by cleavage with *Eco*RI and then mixed with the isolated DNA fragments. Since the fragment and the vector have the same overhanging ends, some of the molecules will hybridize in such a way that the fragment is incorporated into the vector DNA. When the cleavage sites are now closed again using *DNA ligase,* a newly combined *("recombinant") plasmid* arises.

By pretreating a large number of host cells, one can cause some of them to take up the plasmid (a process known as **transformation**) and replicate it along with their own genome when reproducing. To ensure that only host bacteria that contain the plasmid replicate, plasmids are used that give the host *resistance* to a particular antibiotic. When the bacteria are incubated in the presence of this antibiotic, only the cells containing the plasmid will replicate. The plasmid is then isolated from these cells, cleaved with *Eco*RI again, and the fragments are separated using agarose gel electrophoresis (see p. 262). The desired fragment can be identified using its size and then extracted from the gel and used for further experiments.



DNA sequencing

A. Gene libraries ○

It is often necessary in genetic engineering to isolate a DNA segment when its details are not fully known—e.g., in order to determine its nucleotide sequence. In this case, one can use what are known as **DNA libraries**. A DNA library consists of a large number of *vector DNA molecules* containing different fragments of *foreign DNA.* For example, it is possible to take all of the mRNA molecules present in a cell and transcribe them into DNA. These DNA fragments (known as copy DNA or **cDNA**) are then randomly introduced into vector molecules.

A library of genomic DNA can be established by cleaving the total DNA from a cell into small fragments using restriction endonucleases (see p. 258), and then incorporating these into vector DNA. Suitable vectors for gene libraries include **bacteriophages**, for example ("phages" for short). Phages are viruses that only infect bacteria and are replicated by them (see p. 404). Gene libraries have the advantage that they can be searched for specific DNA segments, using hybridization with oligonucleotides.

The first step is to strongly dilute a small part of the library (10^5–10^6 phages in a small volume), mix it with host bacteria, and plate out the mixture onto nutrient medium. The bacteria grow and form a continuous cloudy layer of cells. Bacteria infected by phages grow more slowly. In their surroundings, the bacterial "lawn" is less dense, and a clearer circular zone known as a **plaque** forms. The bacteria in this type of plaque exclusively contain the offspring of a single phage from the library.

The next step is to make an impression of the plate on a plastic foil, which is then heated. This causes the phage DNA to adhere to the foil. When the foil is incubated with a DNA fragment that hybridizes to the DNA segment of interest (a **gene probe**), the probe binds to the sites on the imprint at which the desired DNA is attached. Binding of the gene probe can be detected by prior radioactive or other labeling of the probe. Phages from the positive plaques in the original plate are then isolated and replicated. Restriction cleavage finally provides large amounts of the desired DNA.

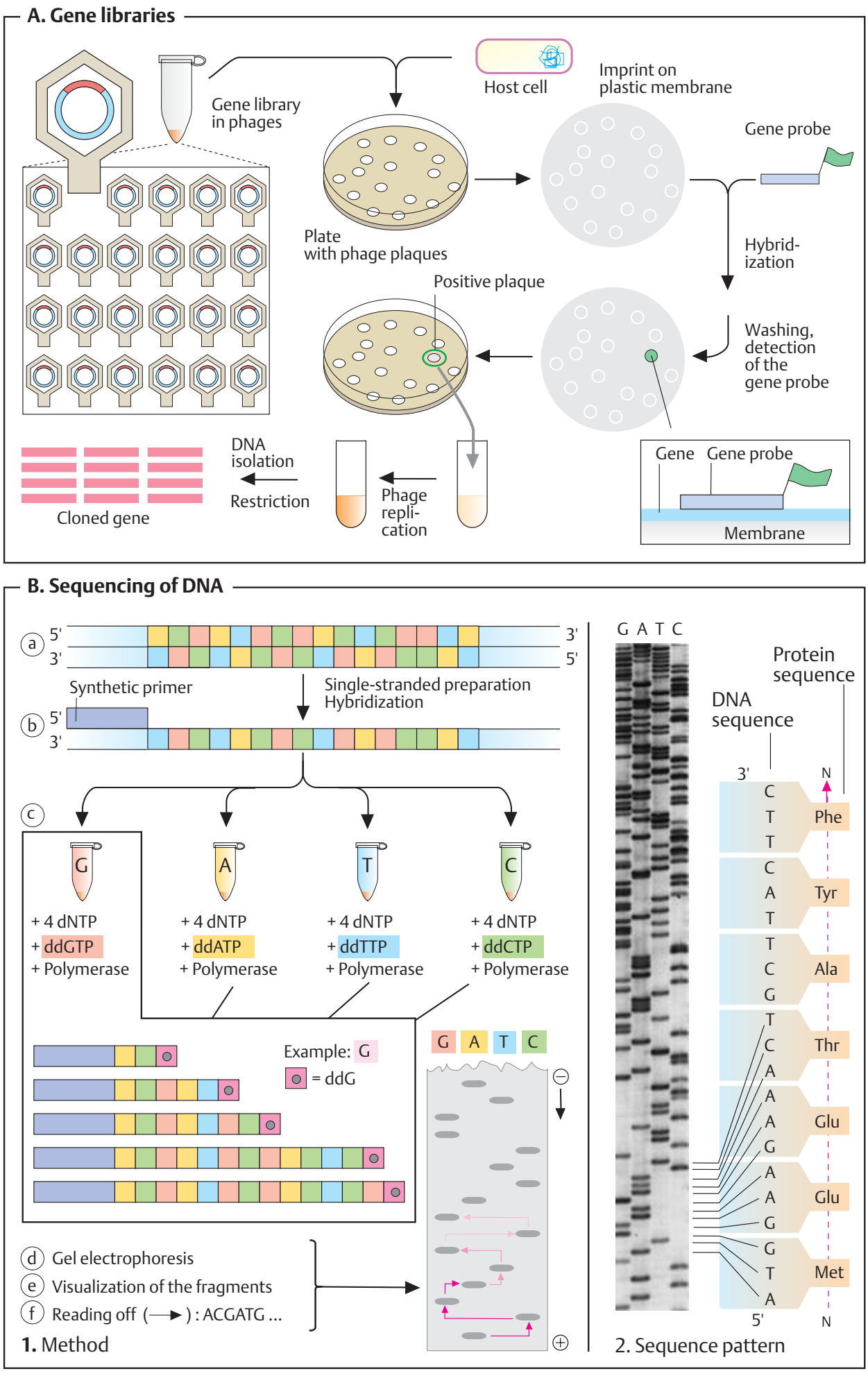
B. Sequencing of DNA ○

The nucleotide sequence of DNA is nowadays usually determined using the so-called **chain termination method**. In single-strand sequencing, the DNA fragment (**a**) is cloned into the DNA of **phage M13** (see p. 404), from which the coded single strand can be easily isolated. This is *hybridized* with a **primer**—a short, synthetically produced DNA fragment that binds to 3′ end of the introduced DNA segment (**b**).

Based on this hybrid, the missing second strand can now be generated in the test tube by adding the four *deoxyribonucleoside triphosphates* (*dNTP*) and a suitable *DNA polymerase* (**c**). The trick lies in also adding small amounts of *dideoxynucleoside triphosphates (ddNTP).* Incorporating a ddNTP leads to the *termination of second-strand synthesis.* This can occur whenever the corresponding dNTP ought to be incorporated. The illustration shows this in detail using the example of ddGTP. In this case, fragments are obtained that each include the primer plus three, six, eight, 13, or 14 additional nucleotides. *Four separate reactions,* each with a different ddNTP, are carried out (**c**), and the products are placed side by side on a supporting material. The fragments are then separated by *gel electrophoresis* (see p. 76), in which they move in relation to their length.

Following *visualization* (**d**), the sequence of the fragments in the individual lanes is simply read from bottom to top (**e**) to directly obtain the nucleotide sequence. A detail from such a sequencing gel and the corresponding protein sequence are shown in Fig. **2**.

In a more modern procedure, the four ddNTPs are covalently marked with fluorescent dyes, which produce a different color for each ddNTP on laser illumination. This allows the sequence in which the individual fragments appear at the lower end of the gel to be continuously recorded and directly stored in digital form.



PCR and protein expression

A. Polymerase chain reaction (PCR) ◑

The polymerase chain reaction (PCR) is an important procedure in genetic engineering that allows any DNA segment to be replicated (**amplified**) without the need for restriction enzymes, vectors, or host cells (see p. 258). However, the nucleotide sequence of the segment has to be known. Two oligonucleotides (**primers**) are needed, which each hybridize with one of the strands at each end of the DNA segment to be amplified; also needed are sufficient quantities of the four **deoxyribonucleoside triphosphates** and a special heat-tolerant DNA polymerase. The primers are produced by chemical synthesis, and the polymerase is obtained from thermostable bacteria.

First, the starter is heated to around 90 °C to separate the DNA double helix into single strands (**a**; cf. p. 84). The mixture is then cooled to allow hybridization of the primers (**b**). Starting from the primers, complementary DNA strands are now synthesized in both directions by the polymerase (**c**). This *cycle* (cycle 1) is *repeated 20–30 times* with the same reaction mixture (cycle 2 and subsequent cycles). The cyclic heating and cooling are carried out by *computer-controlled thermostats.*

After only the third cycle, double strands start to form with a length equal to the distance between the two primers. The proportion of these approximately doubles during each cycle, until almost all of the newly synthesized segments have the correct length.

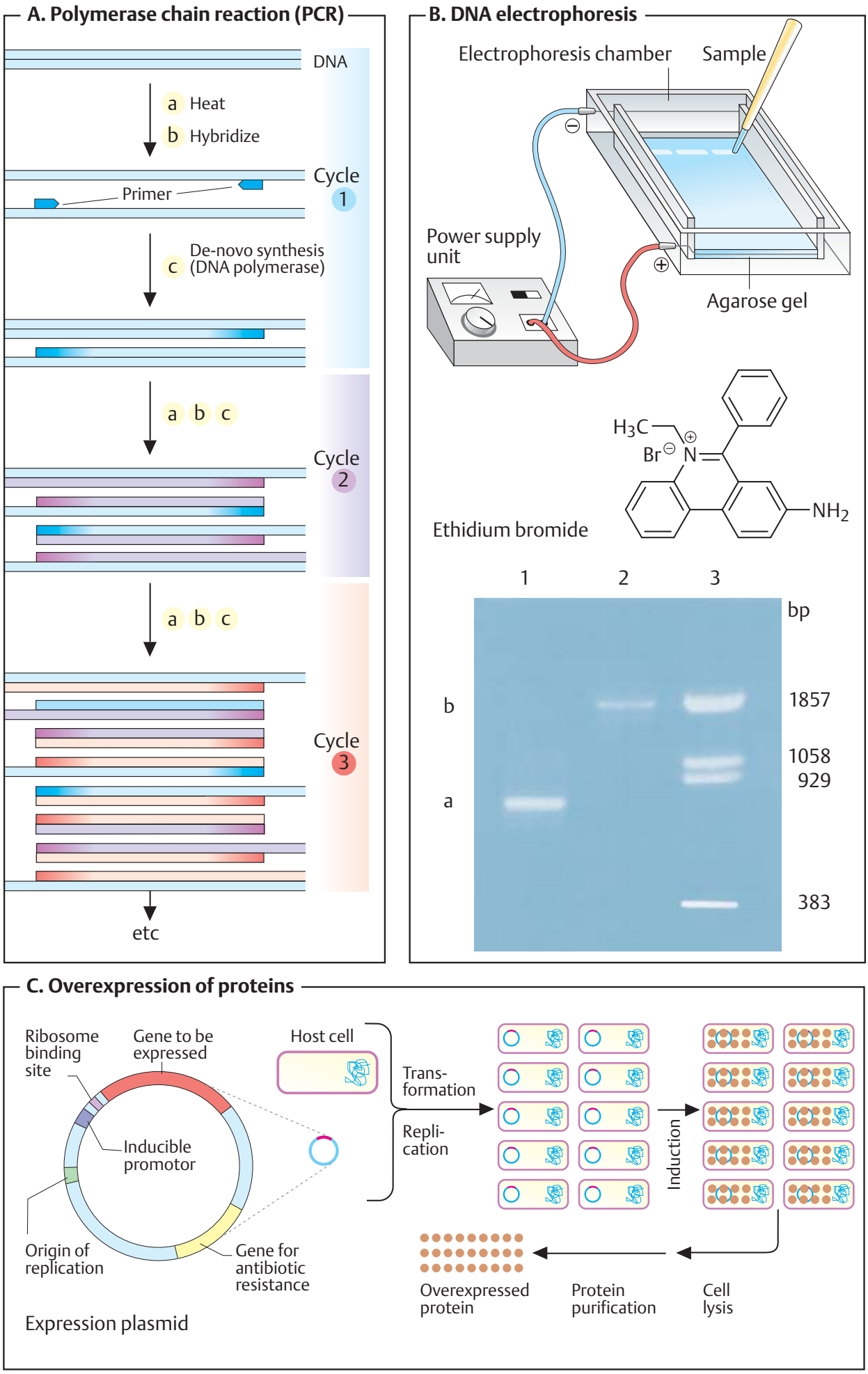
B. DNA electrophoresis ○

The separation of DNA fragments by electrophoresis is technically simpler than protein electrophoresis (see p. 78). The mobility of molecules in an electrical field of a given strength depends on the size and shape of the molecules, as well as their charge. In contrast to proteins, in which all three factors vary, the ratio of mass to charge in nucleic acids is constant, as all of the nucleotide components have similar masses and carry one negative charge. When electrophoresis is carried out in a wide-meshed support material that does not separate according to size and shape, the mobility of the molecules depends on their mass alone. The supporting material generally used in genetic engineering is a gel of the polysaccharide **agarose** (see p. 40). Agarose gels are not very stable and are therefore poured horizontally into a plastic chamber in which they are used for separation (top).

To make the separated fragments visible, after running the procedure the gels are placed in solutions of **ethidium bromide**. This is an intercalator (see p. 254) that shows strong fluorescence in UV light after binding to DNA, although it barely fluoresces in an aqueous solution. The result of separating two PCR amplificates (lanes 1 and 2) is shown in the lower part of the illustration. Comparing their distances with those of polynucleotides of known lengths (lane 3; bp = base pairs) yields lengths of approximately 800 bp for fragment 1 and 1800 bp for fragment 2. After staining, the bands can be cut out of the gel and the DNA can be extracted from them and used for further experiments.

C. Overexpression of proteins ◑

To treat some diseases, proteins are needed that occur in such small quantities in the organism that isolating them on a large scale would not be economically feasible. Proteins of this type can be obtained by *overexpression* in bacteria or eukaryotic cells. To do this, the corresponding *gene* is isolated from human DNA and cloned into an **expression plasmid** as described on p. 258. In addition to the gene itself, the plasmid also has to contain DNA segments that allow replication by the host cell and transcription of the gene. After *transformation* and *replication* of suitable host cells, **induction** is used in a targeted fashion to trigger ef cient *transcription* of the gene. *Translation* of the mRNA formed in the host cell then gives rise to large amounts of the desired protein. Human insulin (see p. 76), plasminogen activators for dissolving blood clots (see p. 292), and the growth hormone somatotropin are among the proteins produced in this way.



Genetic engineering in medicine

Genetic engineering procedures are becoming more and more important in medicine for diagnostic purposes (**A**–**C**). New genetic approaches to the treatment of severe diseases are still in the developmental stage ("gene therapy," **D**).

A. DNA fingerprinting ○

DNA fingerprinting is used to link small amounts of biological material—e. g., traces from the site of a crime—to a specific person. The procedure now used is based on the fact that the human genome contains non-coding repetitive DNA sequences, the length of which varies from individual to individual. **Short tandem repeats** (**STRs**) thus exist in which dinucleotides (e. g., -T-X-) are frequently repeated. Each STR can occur in five to 15 different lengths (alleles), of which one individual possesses only one or two. When the various allele combinations for several STRs are determined after PCR amplification of the DNA being investigated, a "genetic fingerprint" of the individual from whom the DNA originates is obtained. Using comparative material—e. g., saliva samples—definite identification is then possible.

B. Diagnosis of sickle-cell anemia using RFLP ○

This example illustrates a procedure for diagnosing a point mutation in the β-globin gene that leads to sickle-cell anemia (see p. 248). The mutation in the first exon of the gene destroys a cleavage site for the restriction endonuclease *Mst*II (see p. 258). When the DNA of healthy and diseased individuals is cleaved with *Mst*II, different fragments are produced in the region of the β-globin gene, which can be separated by electrophoresis and then demonstrated using specific probes (see p. 260). In addition, heterozygotic carriers of the sickle-cell gene can be distinguished from homozygotic ones.

C. Identification of viral DNA using RT-PCR ○

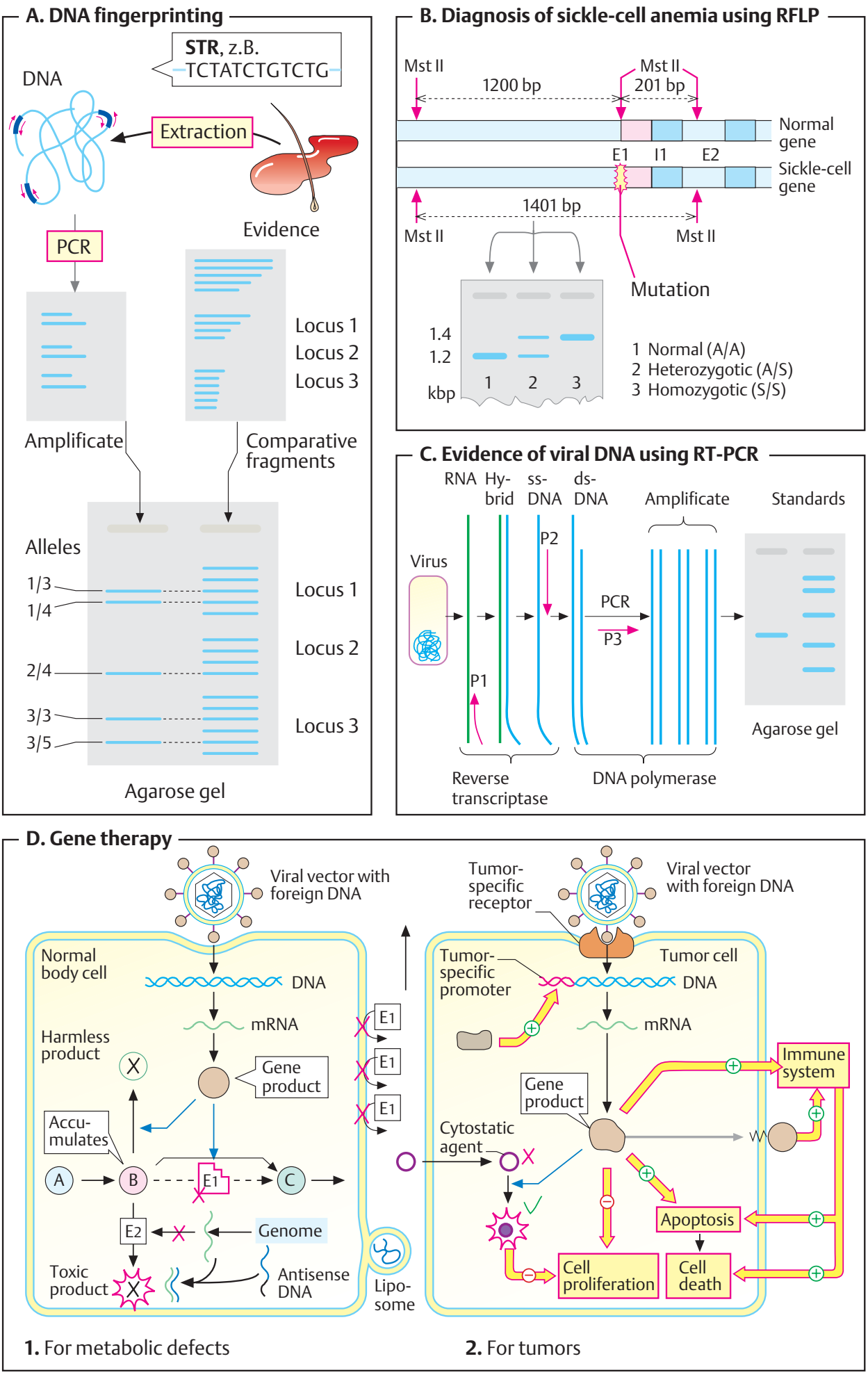
In viral infections, it is often dif cult to determine the species of the pathogen precisely. **RT-PCR** can be used to identify RNA viruses. In this procedure, reverse transcriptase (see p. 404) is used to transcribe the viral RNA into dsDNA, and then PCR is employed to amplify a segment of this DNA with virus-specific primers. In this way, an amplificate with a characteristic length can be obtained for each pathogen and identified using gel electrophoresis as described above.

D. Gene therapy ◑

Many diseases, such as hereditary metabolic defects and tumors, can still not be adequately treated. About 10 years ago, projects were therefore initiated that aimed to treat diseases of this type by transferring genes into the affected cells (gene therapy). The illustration combines conceivable and already implemented approaches to gene therapy for metabolic defects (left) and tumors (right). None of these procedures has yet become established in clinical practice.

If a mutation leads to failure of an enzyme E1 (left), its substrate B will no longer be converted into C and will accumulate. This can lead to cell damage by B itself or by a toxic product formed from it. Treatment with intact E1 is not possible, as the proteins are not capable of passing through the cell membrane. By contrast, it is in principle possible to introduce **foreign genes** into the cell using viruses as vectors (adenoviruses or retroviruses are mainly used). Their gene products could replace the defective E1 or convert B into a harmless product. Another approach uses the so-called **antisense DNA** (bottom right). This consists of polynucleotides that hybridize with the mRNA for specific cellular proteins and thereby prevent their translation. In the case shown, the synthesis of E2 could be blocked, for example.

The main problem in chemotherapy for **tumors** is the lack of tumor-specificity in the highly toxic cytostatic agents used (see p. 402). Attempts are therefore being made to introduce into tumor cells genes with products that are only released from a precursor to form active cytostatics once they have reached their target (left). Other gene products are meant to force the cells into apoptosis (see p. 396) or make them more susceptible to attack by the immune system. To steer the viral vectors to the tumor (targeting), attempts are being made to express proteins on the virus surface that are bound by tumor-specific receptors. Fusion with a tumor-specific promoter could also help limit the effect of the foreign gene to the tumor cells.



Digestion: overview

Most components of food (see p. 360) cannot be resorbed directly by the organism. It is only after they have been broken down into smaller molecules that the organism can take up the essential nutrients. *Digestion* refers to the mechanical and enzymatic breakdown of food and the resorption of the resulting products.

A. Hydrolysis and resorption of food components ●

Following mechanical fragmentation of food during chewing in the mouth, the process of enzymatic degradation starts in the stomach. For this purpose, the chyme is mixed with *digestive enzymes* that occur in the various digestive secretions or in membrane-bound form on the surface of the intestinal epithelium (see p. 268). Almost all digestive enzymes are *hydrolases* (class 3 enzymes; see p. 88); they catalyze the cleavage of composite bonds with the uptake of water.

Proteins are first denatured by the stomach's *hydrochloric acid* (see p. 270), making them more susceptible to attack by the *endopeptidases* (proteinases) present in gastric and pancreatic juice. The peptides released by endopeptidases are further degraded into amino acids by *exopeptidases*. Finally, the amino acids are resorbed by the intestinal mucosa in cotransport with Na^+ ions (see p. 220). There are separate transport systems for each of the various groups of amino acids.

Carbohydrates mainly occur in food in the form of polymers (starches and glycogen). They are cleaved by *pancreatic amylase* into oligosaccharides and are then hydrolyzed by *glycosidases,* which are located on the surface of the intestinal epithelium, to yield monosaccharides. Glucose and galactose are taken up into the enterocytes by secondary active cotransport with Na^+ ions (see p. 220). In addition, monosaccharides also have passive transport systems in the intestine.

Nucleic acids are broken down into their components by *nucleases* from the pancreas and small intestine (ribonucleases and deoxyribonucleases). Further breakdown yields the nucleobases (purine and pyrimidine derivatives), pentoses (ribose and deoxyribose), phosphate, and nucleosides (nucleobase pentose). These cleavage products are resorbed by the intestinal wall in the region of the jejunum.

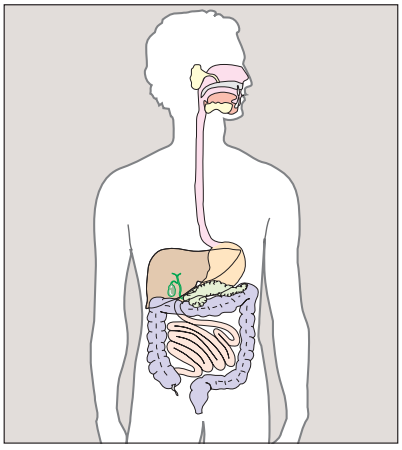
Lipids are a special problem for digestion, as they are not soluble in water. Before enzymatic breakdown, they have to be emulsified by *bile salts* and *phospholipids* in the bile (see p. 314). At the water–lipid interface, *pancreatic lipase* then attacks triacylglycerols with the help of *colipase* (see p. 270). The cleavage products include *fatty acids, 2–monoacylglycerols, glycerol,* and *phosphate* from phospholipid breakdown. After resorption into the epithelial cells, fats are resynthesized from fatty acids, glycerol and 2–monoacylglycerols and passed into the lymphatic system (see p. 272). The lipids in milk are more easily digested, as they are already present in emulsion; on cleavage, they mostly provide short-chain fatty acids.

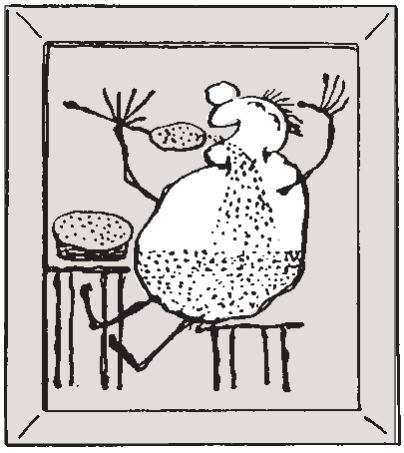
Inorganic components such as water, electrolytes, and *vitamins* are directly absorbed by the intestine.

High-molecular-weight indigestible components, such as the fibrous components of plant cell walls, which mainly consist of cellulose and lignin, pass through the bowel unchanged and form the main component of feces, in addition to cells shed from the intestinal mucosa. Dietary fiber makes a positive contribution to digestion as a *ballast material* by binding water and promoting intestinal peristalsis.

The food components resorbed by the epithelial cells of the intestinal wall in the region of the jejunum and ileum are transported directly to the liver via the *portal vein*. Fats, cholesterol, and lipid–soluble vitamins are exceptions. These are first released by the enterocytes in the form of *chylomicrons* (see p. 278) into the *lymph system,* and only reach the blood via the thoracic duct.

A. Hydrolysis and resorption of dietary constituents





Wilhelm Busch

Nutrients

Proteins

Carbohydrates

Nucleic acids

Lipids

Vitamins
Inorganic substances

Fiber

HCl

Bile salts
Phospholipids

Enzymatic hydrolysis

Cleavage products

Amino acids

Mono-saccharides

Nucleobases
Pentoses
Phosphate
Nucleosides

2-Monoacyl-glycerol
Fatty acids
Cholesterol

Glycerol phosphate

Cellulose
Lignin

Glucose *
Galactose *

*

Resorption

Transport

Hydrophilic

Lipophilic

Portal vessel

Lymph system

Liver

Blood

Feces

* Resorption by active transport

Digestive secretions

A. Digestive juices ◑

Saliva. The salivary glands produce a slightly alkaline secretion which—in addition to water and salts—contains *glycoproteins* (mucins) as lubricants, *antibodies,* and *enzymes.* *α–Amylase* attacks polysaccharides, and a lipase hydrolyzes a small proportion of the neutral fats. α–Amylase and *lysozyme,* a murein-cleaving enzyme (see p. 40), probably serve to regulate the oral bacterial flora rather than for digestion (see p. 340).

Gastric juice. In the stomach, the chyme is mixed with gastric juice. Due to its hydrochloric acid content, this secretion of the gastric mucosa is strongly acidic (pH 1–3; see p. 270). It also contains *mucus* (mainly glycoproteins known as mucins), which protects the mucosa from the hydrochloric acid, *salts,* and *pepsinogen*—the proenzyme ("zymogen") of the aspartate proteinase *pepsin* (see pp. 176, 270). In addition, the gastric mucosa secretes what is known as *"intrinsic factor"*—a glycoprotein needed for resorption of vitamin B_{12} ("extrinsic factor") in the bowel.

In the stomach, pepsin and related enzymes initiate the enzymatic digestion of proteins, which takes 1–3 hours. The acidic gastric contents are then released into the duodenum in batches, where they are neutralized by alkaline pancreatic secretions and mixed with cystic bile.

Pancreatic secretions. In the acinar cells, the pancreas forms a secretion that is alkaline due to its HCO_3^- content, the buffer capacity of which is suf cient to neutralize the stomach's hydrochloric acid. The pancreatic secretion also contains many *enzymes* that catalyze the hydrolysis of high–molecular-weight food components. All of these enzymes are hydrolases with pH optimums in the neutral or weakly alkaline range. Many of them are formed and secreted as proenzymes and are only activated in the bowel lumen (see p. 270).

Trypsin, chymotrypsin, and *elastase* are endopeptidases that belong to the group of serine proteinases (see p. 176). Trypsin hydrolyzes specific peptide bonds on the C side of the basic amino acids Arg and Lys, while chymotrypsin prefers peptide bonds of the apolar amino acids Tyr, Trp, Phe, and Leu (see p. 94). Elastase mainly cleaves on the C side of the aliphatic amino acids Gly, Ala, Val, and Ile. Smaller peptides are attacked by *carboxypeptidases,* which as exopeptidases cleave individual amino acids from the C–terminal end of the peptides (see p. 176).

~–Amylase, the most important endoglycosidase in the pancreas, catalyzes the hydrolysis of α1→4 bonds in the polymeric carbohydrates starch and glycogen. This releases maltose, maltotriose, and a mixture of other oligosaccharides.

Various pancreatic enzymes hydrolyze lipids, including *lipase* with its auxiliary protein *colipase* (see p. 270), *phospholipase* A_2, and *sterol esterase.* Bile salts activate the lipid-cleaving enzymes through micelle formation (see below).

Several hydrolases—particularly *ribonuclease* (RNAse) and *deoxyribonuclease* (DNAse)—break down the nucleic acids contained in food.

Bile. The liver forms a thin secretion (bile) that is stored in the gallbladder after water and salts have been extracted from it. From the gallbladder, it is released into the duodenum. The most important constituents of bile are *water* and inorganic *salts, bile acids* and *bile salts* (see p. 314), *phospholipids, bile pigments,* and *cholesterol.* Bile salts, together with phospholipids, emulsify insoluble food lipids and activate the lipases. Without bile, fats would be inadequately cleaved, if at all, resulting in "fatty stool" (steatorrhea). Resorption of fat-soluble vitamins would also be affected.

Small-intestinal secretions. The glands of the small intestine (the Lieberkühn and Brunner glands) secrete additional digestive enzymes into the bowel. Together with enzymes on the microvilli of the intestinal epithelium (peptidases, glycosidases, etc.), these enzymes ensure almost complete hydrolysis of the food components previously broken down by the endoenzymes.

A. Digestive juices

Saliva

Daily secretion 1.0–1.5 l
pH 7

Water	Moistens food
Salts	
Mucus	Lubricant
Antibodies	Bind to bacteria
α-Amylases (3.2.1.1)	Cleave starch ↓
Lysozyme (3.2.1.17)	Attacks bacterial cell walls ↓

Bile

Daily secretion 0.6 l
pH 6.9–7.7

Water	
HCO_3^-	Neutralizes gastric juice
Bile salts	Facilitate lipid digestion
Phospholipids	Facilitate lipid digestion
Bile pigments	Waste products
Cholesterol	Waste product

↓ Endoenzyme
-◂ Exoenzyme

Compo-nents | Function or substrate

Gastric juice

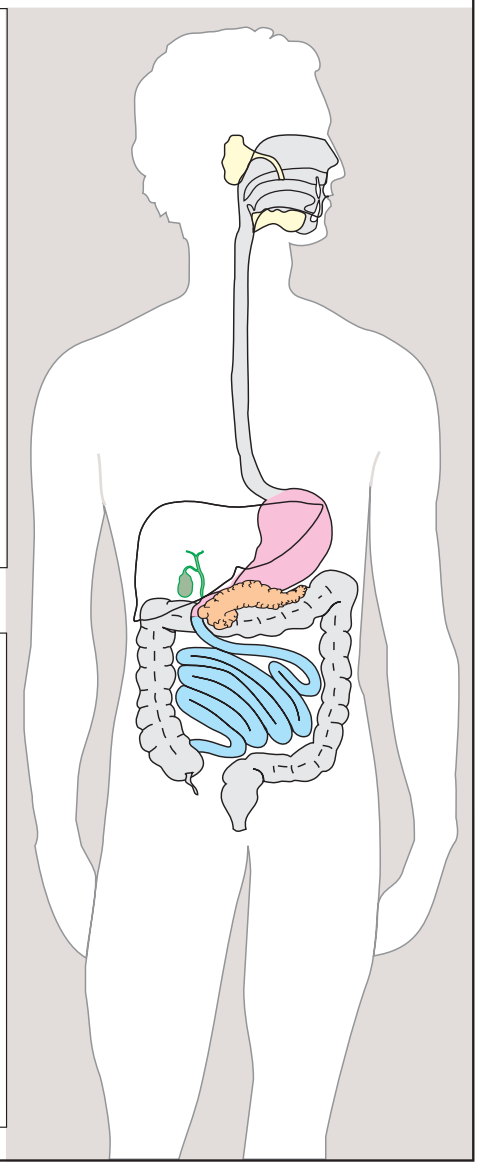
Daily secretion 2–3 l
pH 1

Water	
Salts	
HCl	Denatures proteins, kills bacteria
Mucus	Protects stomach lining
Pepsins (3.4.23.1-3)	Cleave proteins ↓
Chymosin (3.4.23.4)	Precipitates casein ↓
Triacylglycerol lipase (3.1.1.3)	Cleaves fats
Intrinsic factor	Protects vitamin B_{12}

Pancreatic secretions

Daily secretion 0.7–2.5 l
pH 7.7 (7.5–8.8)

Water	
HCO_3^-	Neutralizes gastric juice
Trypsin (3.4.21.4)	Proteins ↓
Chymotrypsin (3.4.21.1)	Proteins ↓
Elastase (3.4.21.36)	Proteins ↓
Carboxypeptidases (3.4.n.n)	Peptides -◂
α-Amylase (3.2.1.1)	Starch and glycogen ↓
Triacylglycerol lipase (3.1.1.3)	Fats
Co-Lipase	Cofactor for lipase
Phospholipase A_2 (3.1.1.4)	Phospholipids
Sterol esterase (3.1.1.13)	Cholesterol esters
Ribonuclease (3.1.27.5)	RNA
Deoxyribonuclease I (3.1.21.1)	DNA



Secretions of the small intestine

Daily secretion unknown
pH 6.5–7.8

Aminopeptidases (3.4.11.n)	Peptides -◂
Dipeptidases (3.4.13.n)	Dipeptides
α-Glucosidase (3.2.1.20)	Oligosaccharides ↓
Oligo-1,6-glucosidase (3.2.1.10)	Oligosaccharides ↓
β-Galactosidase (3.2.1.23)	Lactose
Sucrose α-glucosidase (3.2.1.48)	Sucrose
α, α-Trehalase (3.2.1.28)	Trehalose
Alkaline phosphatase (3.1.3.1)	Phosphoric acid esters
Polynucleotidases (3.1.3.n)	Nucleic acids, nucleotides ↓
Nucleosidases (3.2.2.n)	Nucleosides -◂
Phospholipases (3.1.n.n)	Phospholipids

Digestive processes

Gastric juice is the product of several cell types. The *parietal cells* produce hydrochloric acid, *chief cells* release pepsinogen, and *accessory cells* form a mucin-containing mucus.

A. Formation of hydrochloric acid ◑

The secretion of **hydrochloric acid** (H^+ and Cl^-) by the parietal cells is an active process that uses up ATP and takes place against a concentration gradient (in the gastric lumen, with a pH of 1, the H^+ concentration is some 10^6 times higher than in the parietal cells, which have a pH of 7).

The precursors of the exported H^+ ions are carbon dioxide (CO_2) and water (H_2O). CO_2 diffuses from the blood into the parietal cells, and in a reaction catalyzed by *carbonate dehydratase* (carbonic anhydrase [2]), it reacts with H_2O to form H^+ and hydrogen carbonate (HCO_3^-). The H^+ ions are transported into the gastric lumen in exchange for K^+ by a membrane-bound *H^+/K^+-exchanging ATPase* [1] (a transport ATPase of the P type; see p. 220). The remaining hydrogen carbonate is released into the interstitium in electroneutral antiport in exchange for chloride ions (Cl^-), and from there into the blood. The Cl^- ions follow the secreted protons through a channel into the gastric lumen.

The hydrochloric acid in gastric juice is important for digestion. It activates pepsinogen to form pepsin (see below) and creates an optimal pH level for it to take effect. It also denatures food proteins so that they are more easily attacked by proteinases, and it kills micro-organisms.

Regulation. HCl secretion is stimulated by the peptide hormone *gastrin,* the mediator *histamine* (see p. 380), and—via the neurotransmitter *acetylcholine*—by the autonomous nervous system. The peptide *somatostatin* and certain *prostaglandins* (see p. 390) have inhibitory effects. Together with cholecystokinin, secretin, and other peptides, gastrin belongs to the group of **gastrointestinal hormones** (see p. 370). All of these are formed in the gastrointestinal tract and mainly act in the vicinity of the site where they are formed—i. e., they are paracrine hormones (see p. 372). While gastrin primarily enhances HCl secretion, *cholecystokinin* and *secretin* mainly stimulate pancreatic secretion and bile release.

B. Zymogen activation ◑

To prevent self–digestion, the pancreas releases most proteolytic enzymes into the duodenum in an inactive form as *proenzymes* (zymogens). Additional protection from the effects of premature activation of pancreatic proteinases is provided by *proteinase inhibitors* in the pancreatic tissue, which inactivate active enzymes by complex formation (right).

Trypsinogen plays a key role among the proenzymes released by the pancreas. In the bowel, it is proteolytically converted into active trypsin (see p. 176) by **enteropeptidase**, a membrane enzyme on the surface of the enterocytes. Trypsin then autocatalytically activates additional trypsinogen molecules and the other proenzymes (left).

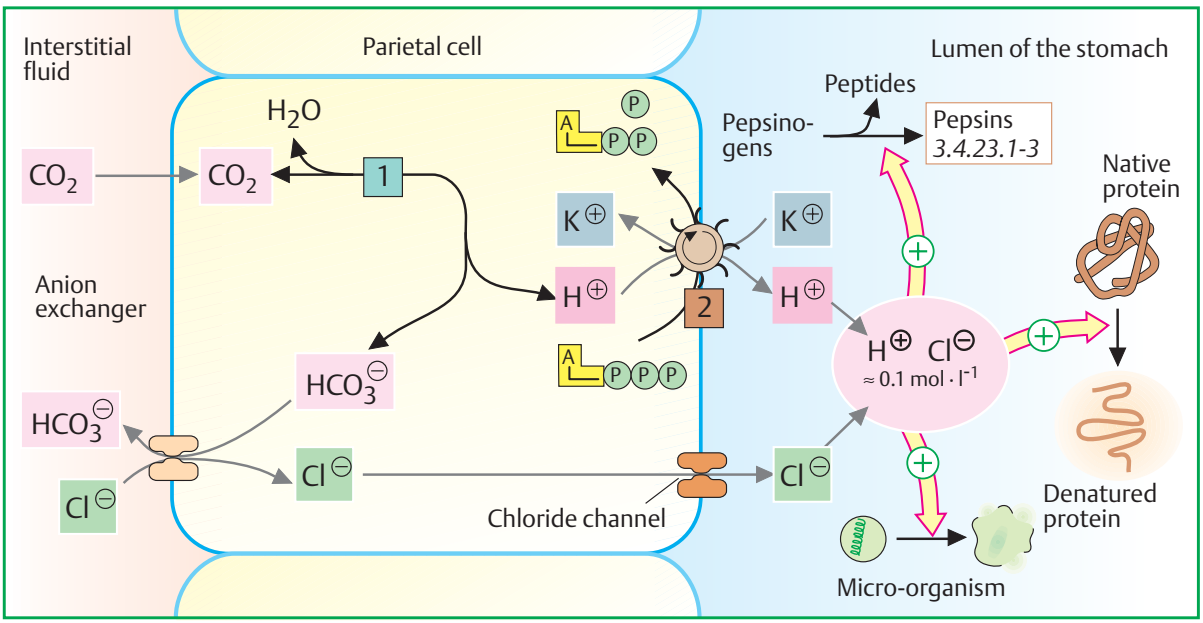
C. Fat digestion ◑

Due to the "hydrophobic effect" (see p. 28), water-insoluble neutral fats in the aqueous environment of the bowel lumen would aggregate into drops of fat in which most of the molecules would not be accessible to pancreatic lipase. The amphipathic substances in bile (bile acids, bile salts, phospholipids) create an **emulsion** in which they occupy the surface of the droplets and thereby prevent them from coalescing into large drops. In addition, the bile salts, together with the auxiliary protein colipase, mediate binding of *triacylglycerol lipase* [1] to the emulsified fat droplets. Activation of the lipase is triggered by a conformation change in the C-terminal domain of the enzyme, which uncovers the active center.

During passage through the intestines, the active lipase breaks down the triacylglycerols in the interior of the droplets into free fatty acids and amphipathic monoacylglycerols. Over time, smaller **micelles** develop (see p. 28), in the envelope of which monoacylglycerols are present in addition to bile salts and phospholipids. Finally, the components of the micelles are resorbed by the enterocytes in ways that have not yet been explained.

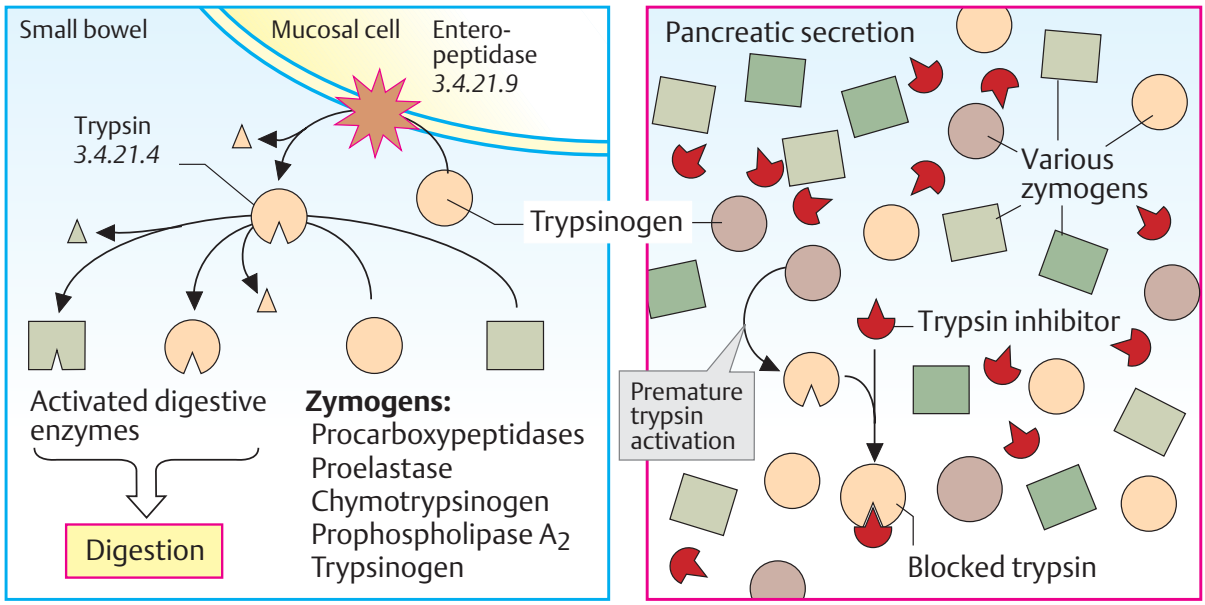
Monoacylglycerols and fatty acids are reassembled into fats again (see p. 272), while the bile acids return to the liver (enterohepatic circulation; see p. 314).

A. Formation of hydrochloric acid

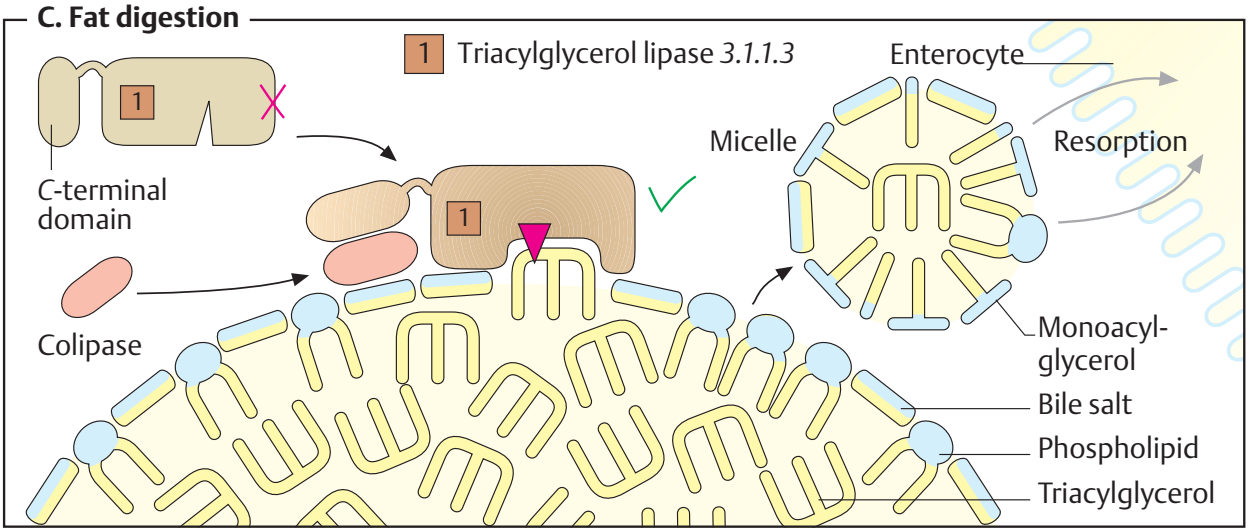


1 Carbonate dehydratase *4.2.1.1* [Zn^{2+}] 2 H^+/K^+-exchanging ATPase *3.6.1.36*

B. Zymogen activation



C. Fat digestion



Resorption

Enzymatic hydrolysis in the digestive tract breaks down foodstuffs into their resorbable components. *Resorption* of the cleavage products takes place primarily in the small intestine. Only ethanol and short–chain fatty acids are already resorbed to some extent in the stomach.

The resorption process is facilitated by the large inner surface of the intestine, with its brush–border cells. Lipophilic molecules penetrate the plasma membrane of the mucosal cells by simple diffusion, whereas polar molecules require *transporters* (facilitated diffusion; see p. 218). In many cases, carrier-mediated cotransport with Na^+ ions can be observed. In this case, the difference in the concentration of the sodium ions (high in the intestinal lumen and low in the mucosal cells) drives the import of nutrients against a concentration gradient (secondary active transport; see p. 220). Failure of carrier systems in the gastrointestinal tract can result in diseases.

A. Monosaccharides ◑

The cleavage of polymeric carbohydrates by *~–amylase* [1] leads to **oligosaccharides**, which are broken down further by *exoglycosidases* (oligosaccharidases and disaccharidases [2]) on the membrane surface of the brush border. The monosaccharides released in this way then pass with the help of various *sugar–specific transporters* into the cells of intestinal epithelium. *Secondary active transport* serves for the uptake of **glucose** and **galactose**, which are transported against a concentration gradient in cotransport with Na^+. The Na^+ gradient is maintained on the basal side of the cells by *Na^+/K^+-ATPase* [3]. Another passive transporter then releases glucose and galactose into the blood. **Fructose** is taken up by a special type of transporter using facilitated diffusion.

Amino acids (not illustrated)

Protein degradation is initiated by *proteinases*—by pepsins in the stomach and by trypsin, chymotrypsin, and elastase in the small intestine. The resulting peptides are then further hydrolyzed by various *peptidases* into amino acids. Individual amino acid groups have *group–specific amino acid transporters,* some of which transport the amino acids into the enterocytes in cotransport with Na^+ ions (secondary active transport), while others transport them in an Na^+–independent manner through facilitated diffusion. Small peptides can also be taken up.

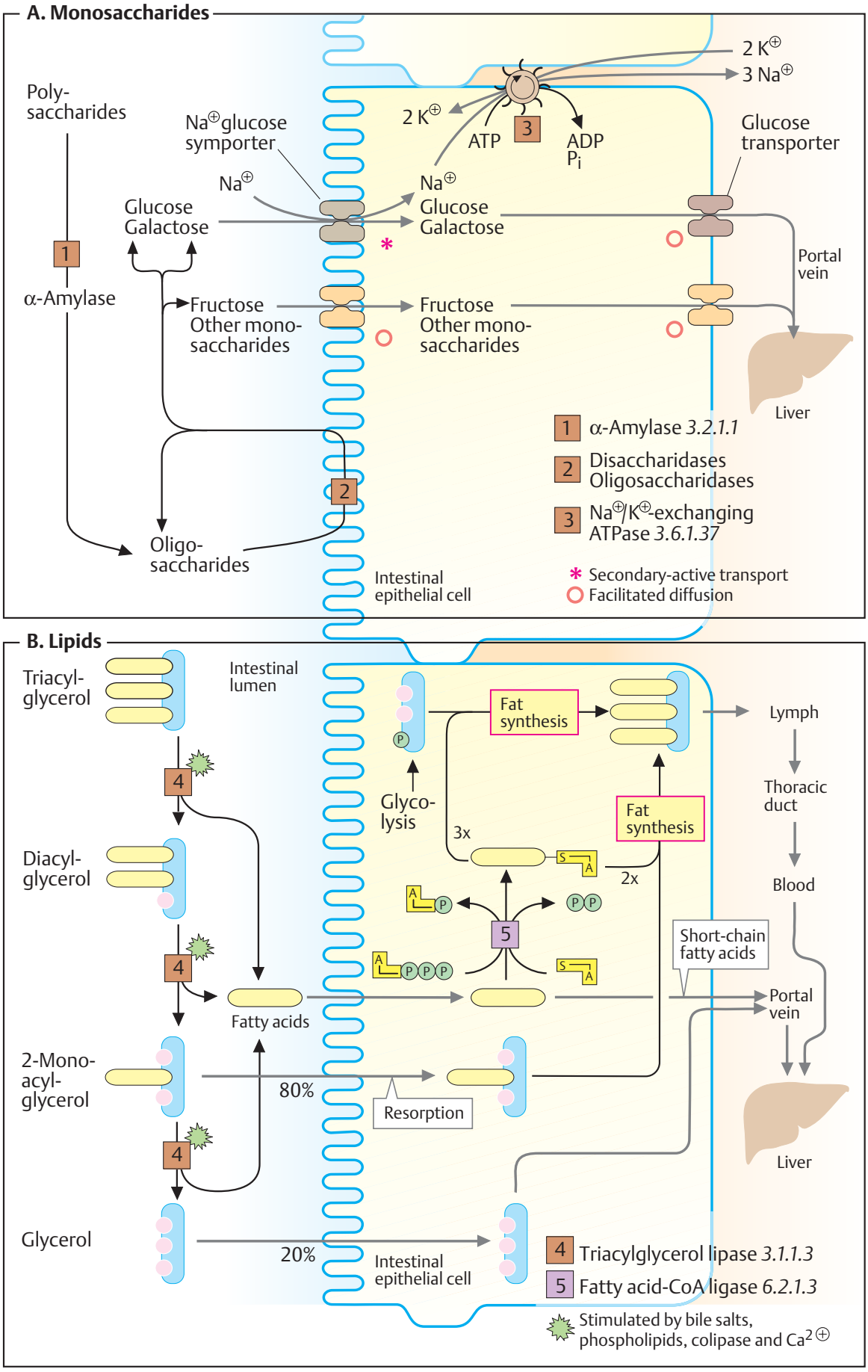
B. Lipids ◑

Fats and other lipids are poorly soluble in water. The larger the accessible surface is—i. e., the better the fat is emulsified—the easier it is for enzymes to hydrolyze it (see p. 270). Due to the special properties of milk, milk fats already reach the gastrointestinal tract in emulsified form. Digestion of them therefore already starts in the oral cavity and stomach, where lipases in the saliva and gastric juice are available. Lipids that are less accessible—e. g., from roast pork—are emulsified in the small intestine by *bile salts* and *bile phospholipids.* Only then are they capable of being attacked by *pancreatic lipase* [4] (see p. 270).

Fats (triacylglycerols) are mainly attacked by pancreatic lipase at positions 1 and 3 of the glycerol moiety. Cleavage of two fatty acid residues gives rise to **fatty acids** and **2-monoacylglycerols**, which are quantitatively the most important products. However, a certain amount of **glycerol** is also formed by complete hydrolysis. These cleavage products are resorbed by a non-ATP-dependent process that has not yet been explained in detail.

In the mucosal cells, *long-chain fatty acids* are resynthesized by an ATP-dependent ligase [5] to form acyl-CoA and then triacylglycerols (fats; see p. 170). The fats are released into the lymph in the form of **chylomicrons** (see p. 278) and, bypassing the liver, are deposited in the thoracic duct—i. e., the blood system. *Cholesterol* also follows this route.

By contrast, *short-chain fatty acids* (with chain lengths of less than 12 C atoms) pass directly into the blood and reach the liver via the portal vein. Resorbed glycerol can also take this path.



Blood: composition and functions

Human blood constitutes about 8% of the body's weight. It consists of **cells** and cell fragments in an aqueous medium, the **blood plasma.** The proportion of cellular elements, known as *hematocrit,* in the total volume is approximately 45%.

A. Functions of the blood ●

The blood is the most important transport medium in the body. It serves to keep the "internal milieu" constant (homeostasis) and it plays a decisive role in defending the body against pathogens.

Transport. The *gases* oxygen and carbon dioxide are transported in the blood. The blood mediates *the exchange of substances between organs* and takes up *metabolic end products* from tissues in order to transport them to the lungs, liver, and kidney for excretion. The blood also distributes *hormones* throughout the organism (see p. 370).

Homeostasis. The blood ensures that a balanced distribution of water is maintained between the vascular system, the cells (intracellular space), and the extracellular space. The *acid–base balance* is regulated by the blood in combination with the lungs, liver, and kidneys (see p. 288). The regulation of *body temperature* also depends on the controlled transport of heat by the blood.

Defense. The body uses both non-specific and specific mechanisms to defend itself against pathogens. The defense system includes the *cells of the immune system* and certain *plasma proteins* (see p. 294).

Self-protection. To prevent blood loss when a vessel is injured, the blood has systems for stanching blood flow and coagulating the blood (hemostasis; see p. 290). The dissolution of blood clots (fibrinolysis) is also managed by the blood itself (see p. 292).

B. Cellular elements ◑

The solid elements in the blood are the erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets).

The **erythrocytes** provide for gas transport in the blood. They are discussed in greater detail on pp. 280–285.

The **leukocytes** include various types of granulocyte, monocyte, and lymphocyte. All of these have immune defense functions (see p. 294). The *neutrophil granulocytes, monocytes,* and the *macrophages* derived from monocytes are phagocytes. They can ingest and degrade invading pathogens. The *lymphocytes* are divided into two groups, B lymphocytes and T lymphocytes. B lymphocytes produce *antibodies,* while T lymphocytes regulate the immune response and destroy virus-infected cells and tumor cells. *Eosinophilic* and *basophilic granulocytes* have special tasks for defense against animal parasites.

Thrombocytes are cell fragments that arise in the bone marrow from large precursor cells, the megakaryocytes. Their task is to promote hemostasis (see p. 290).

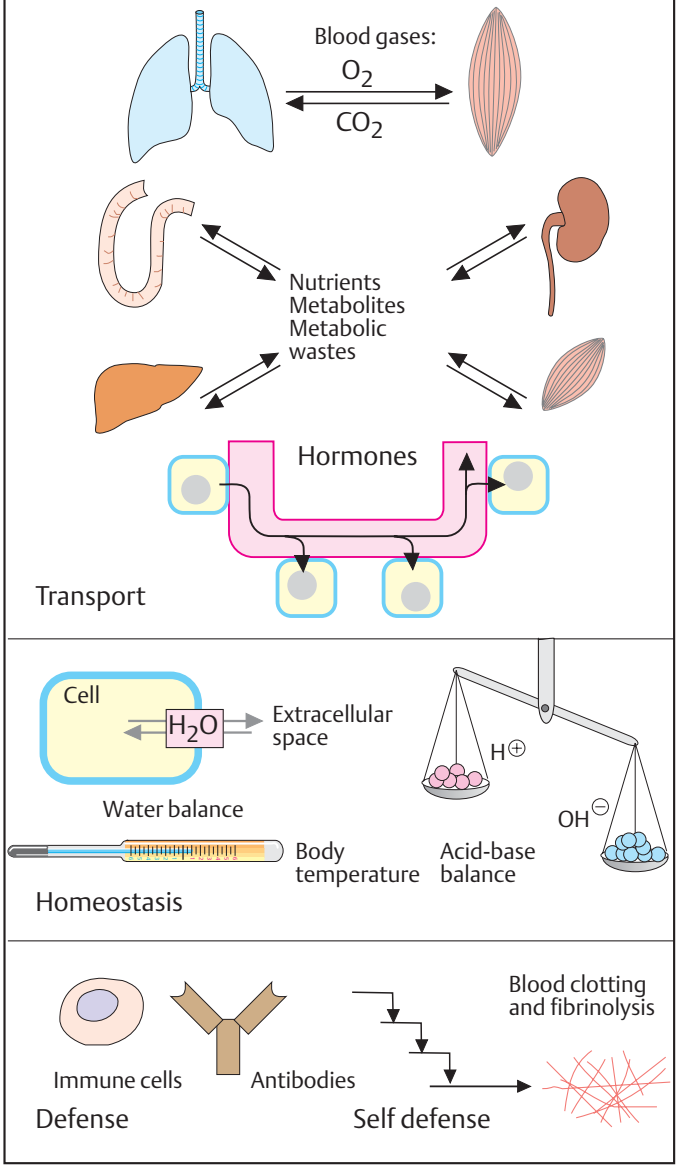
C. Blood plasma: composition ◑

The **blood plasma** is an aqueous solution of electrolytes, nutrients, metabolites, proteins, vitamins, trace elements, and signaling substances. The fluid phase of coagulated blood is known as **blood serum**. It differs from the plasma in that it lacks fibrin and other coagulation proteins (see p. 290).

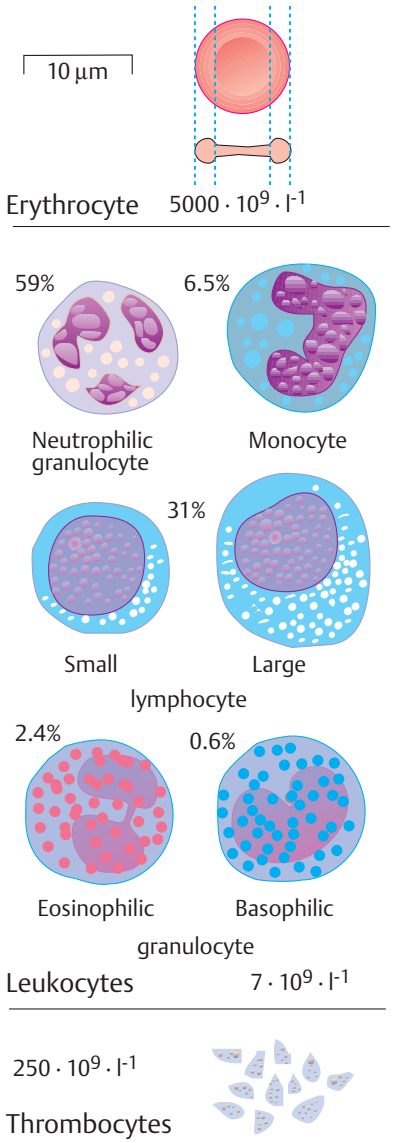
Laboratory assessment of the composition of the blood plasma is often carried out in clinical chemistry. Among the electrolytes, there is a relatively high concentration of Na^+, Ca^{2+}, and Cl^- ions in the blood in comparison with the cytoplasm. By contrast, the concentrations of K^+, Mg^{2+}, and phosphate ions are higher in the cells. Proteins also have a higher intracellular concentration. The electrolyte composition of blood plasma is similar to that of seawater, due to the evolution of early forms of life in the sea. The solution known as *"physiological saline"* (NaCl at a concentration of 0.15 mol L^{-1}) is almost isotonic with blood plasma.

A list of particularly important **metabolites** in the blood plasma is given on the right.

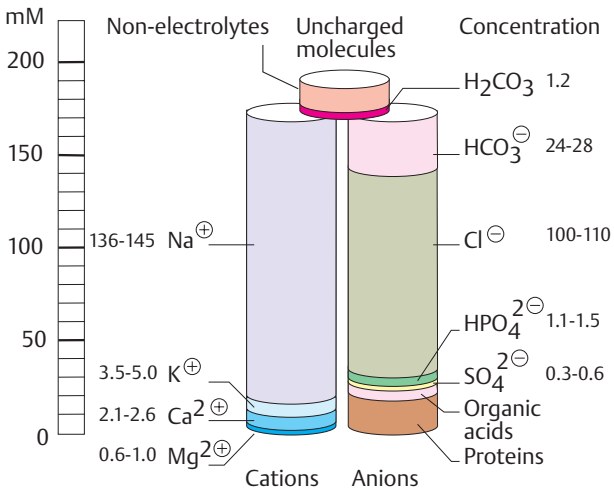
A. Functions of the blood



B. Cellular elements



C. Blood plasma: composition



Metabolite	Concentration (mM)
Glucose	3.6 – 6.1
Lactate	0.4 – 1.8
Pyruvate	0.07 – 0.11
Urea	3.5 – 9.0
Uric acid	0.18 – 0.54
Creatinin	0.06 – 0.13
Amino acids	2.3 – 4.0
Ammonia	0.02 – 0.06
Lipids (total)	5.5 – 6.0 $g \cdot l^{-1}$
Triacylglycerols	1.0 – 1.3 $g \cdot l^{-1}$
Cholesterol	1.7 – 2.1 $g \cdot l^{-1}$

Plasma proteins

Quantitatively, proteins are the most important part of the soluble components of the blood plasma. With concentrations of between 60 and 80 g L^{-1}, they constitute approximately 4% of the body's total protein. Their tasks include transport, regulation of the water balance, hemostasis, and defense against pathogens.

A. Plasma proteins ◑

Some 100 different proteins occur in human blood plasma. Based on their behavior during electrophoresis (see below), they are broadly divided into *five fractions:* **albumins** and α_1–, α_2–, β– and γ-**globulins**. Historically, the distinction between the albumins and globulins was based on differences in the proteins' solubility –albumins are soluble in pure water, whereas globulins only dissolve in the presence of salts.

The most frequent protein in the plasma, at around 45 g L^{-1}, is **albumin**. Due to its high concentration, it plays a crucial role in maintaining the blood's colloid osmotic pressure and represents an important amino acid reserve for the body. Albumin has binding sites for apolar substances and therefore functions as a transport protein for long-chain fatty acids, bilirubin, drugs, and some steroid hormones and vitamins. In addition, serum albumin binds Ca^{2+} and Mg^{2+} ions. It is the only important plasma protein that is not glycosylated.

The albumin fraction also includes *transthyretin* (prealbumin), which together with other proteins transports the hormone thyroxine and its metabolites.

The table also lists important **globulins** in blood plasma, with their mass and function. The α- and β-globulins are involved in the transport of lipids (lipoproteins; see p. 278), hormones, vitamins, and metal ions. In addition, they provide coagulation factors, protease inhibitors, and the proteins of the complement system (see p. 298). Soluble antibodies (immunoglobulins; see p. 300) make up the γ-globulin fraction.

Synthesis and degradation. Most plasma proteins are synthesized by the liver. Exceptions to this include the immunoglobulins, which are secreted by B lymphocytes known as plasma cells (see p. 302) and peptide hormones, which derive from endocrine gland cells.

With the exception of albumin, almost all plasma proteins are *glycoproteins.* They carry oligosaccharides in *N*-and *O*-glycosidic bonds (see p. 44). *N*-acetylneuraminic acid (sialic acid; see p. 38) often occurs as a terminal carbohydrate among sugar residues. *Neuraminidases* (sialidases) on the surface of the vascular endothelia gradually cleave the sialic acid residues and thereby release galactose units on the surfaces of the proteins. These *asialoglycoproteins* ("asialo-" = without sialic acid) are recognized and bound by galactose receptors on hepatocytes. In this way, the liver takes up aged plasma proteins by endocytosis and breaks them down. The oligosaccharides on the protein surfaces thus determine the half-life of plasma proteins, which is a period of days to weeks.

In healthy individuals, the concentration of plasma proteins is constant. Diseases in organs that are involved in protein synthesis and breakdown can shift the protein pattern. For example, via cytokines (see p. 392), severe injuries trigger increased synthesis of *acute-phase proteins,* which include C-reactive protein, haptoglobin, fibrinogen, complement factor C-3, and others. The concentrations of individual proteins are altered in some diseases (known as *dysproteinemias*).

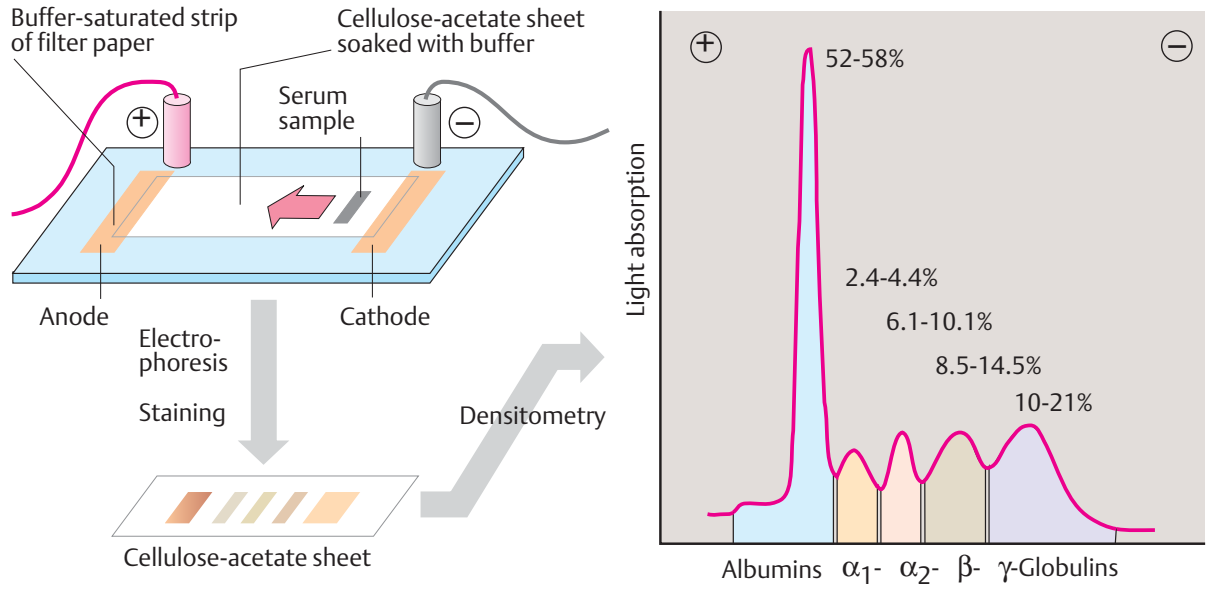
B. Carrier electrophoresis ◑

Proteins and other electrically charged macromolecules can be separated using electrophoresis (see also pp. 78, 262). Among the various procedures used, **carrier electrophoresis** on cellulose acetate foil (CAF) is particularly simple. Using this method, serum proteins—which at slightly alkaline pH values all move towards the anode, due to their excess of negative charges—can be separated into the five fractions mentioned. After the proteins have been stained with dyes, the resulting bands can be quantitatively assessed using densitometry.

A. Plasma proteins

Group	Protein	M_r in kDa	Function
Albumins:	Transthyretin	50-66	Transport of thyroxin and triiodothyronin
	Albumin: 45 g · l^{-1}	67	Maintenance of osmotic pressure; transport of fatty acids, bilirubin, bile acids, steroid hormones, pharmaceuticals and inorganic ions.
α_1-Globulins:	Antitrypsin	51	Inhibition of trypsin and other proteases
	Antichymotrypsin	58-68	Inhibition of chymotrypsin
	Lipoprotein (HDL)	200-400	Transport of lipids
	Prothrombin	72	Coagulation factor II, thrombin precursor (*3.4.21.5*)
	Transcortin	51	Transport of cortisol, corticosterone and progesterone
	Acid glycoprotein	44	Transport of progesterone
	Thyroxin-binding globulin	54	Transport of iodothyronins
α_2-Globulins:	Ceruloplasmin	135	Transport of copper ions
	Antithrombin III	58	Inhibition of blood clotting
	Haptoglobin	100	Binding of hemoglobin
	Cholinesterase (3.1.1.8)	ca. 350	Cleavage of choline esters
	Plasminogen	90	Precursor of plasmin (*3.4.21.7*), breakdown of blood clots
	Macroglobulin	725	Binding of proteases, transport of zinc ions
	Retinol-binding protein	21	Transport of vitamin A
	Vitamin D-binding protein	52	Transport of calciols
β-Globulins:	Lipoprotein (LDL)	2.000-4.500	Transport of lipids
	Transferrin	80	Transport of iron ions
	Fibrinogen	340	Coagulation factor I
	Sex hormone-binding globulin	65	Transport of testosterone and estradiol
	Transcobalamin	38	Transport of vitamin B_{12}
	C-reactive protein	110	Complement activation
γ-Globulins:	IgG	150	Late antibodies
	IgA	162	Mucosa-protecting antibodies
	IgM	900	Early antibodies
	IgD	172	B-lymphocyte receptors
	IgE	196	Reagins

B. Electrophoresis



Lipoproteins

Most lipids are barely soluble in water, and many have amphipathic properties. In the blood, free triacylglycerols would coalesce into drops that could cause fat embolisms. By contrast, amphipathic lipids would be deposited in the blood cells' membranes and would dissolve them. Special precautions are therefore needed for lipid transport in the blood. While long-chain fatty acids are bound to albumin and short-chain ones are dissolved in the plasma (see p. 276), other lipids are transported in **lipoprotein complexes**, of which there several types in the blood plasma, with different sizes and composition.

A. Composition of lipoprotein complexes ◑

Lipoproteins are spherical or discoid aggregates of **lipids** and **apoproteins**. They consist of a nucleus of apolar lipids (triacylglycerols and cholesterol esters) surrounded by a single-layered shell approximately 2 nm thick of amphipathic lipids (phospholipids and cholesterol; the example shown here is LDL). The shell, in which the apoproteins are also deposited, gives the surfaces of the particles polar properties and thereby prevents them from aggregating into large particles. The larger the lipid nucleus of a lipoprotein is—i. e., the larger the number of apolar lipids it contains—the lower its density is.

Lipoproteins are classified into five groups. In order of decreasing size and increasing density, these are: *chylomicrons, VLDLs* (very-low-density lipoproteins), *IDLs* (intermediate-density lipoproteins), *LDLs* (low-density lipoproteins), and *HDLs* (high-density lipoproteins). The proportions of apoproteins range from 1% in chylomicrons to over 50% in HDLs. These proteins serve less for solubility purposes, but rather function as recognition molecules for the membrane receptors and enzymes that are involved in lipid exchange.

B. Transport functions

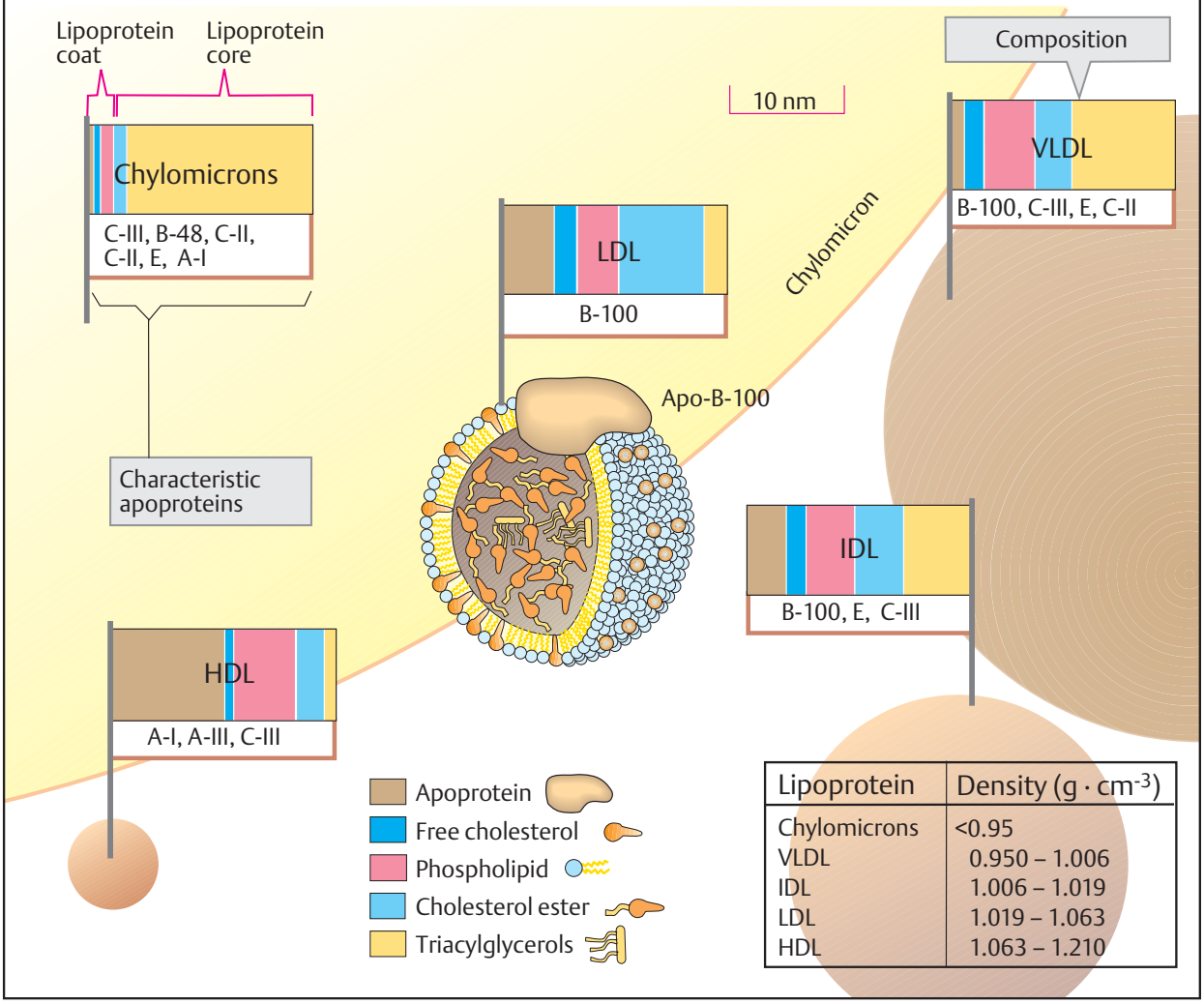
The classes of lipoproteins differ not only in their composition, but also in the ways in which they originate and function.

The **chylomicrons** take care of the transport of triacylglycerols from the intestine to the tissues. They are formed in the intestinal mucosa and reach the blood via the lymphatic system (see p. 266). In the peripheral vessels—particularly in muscle and adipose tissue—*lipoprotein lipase* [1] on the surface of the vascular endothelia hydrolyzes most of the triacylglycerols. Chylomicron breakdown is activated by the transfer of apoproteins E and C from HDL. While the fatty acids released and the glycerol are taken up by the cells, the chylomicrons gradually become converted into **chylomicron remnants**, which are ultimately removed from the blood by the liver.

VLDLs, **IDLs**, and **LDLs** are closely related to one another. VLDLs formed in the liver (see p. 312) transport triacylglycerols, cholesterol, and phospholipids to other tissues. Like chylomicrons, they are gradually converted into IDL and LDL under the influence of *lipoprotein lipase* [1]. This process is also stimulated by HDL. Cells that have a demand for cholesterol bind LDL through an interaction between their **LDL receptor** and ApoB-100, and then take up the complete particle through **receptor-mediated endocytosis**. This type of transport is mediated by depressions in the membrane (*"coated pits"*), the interior of which is lined with the protein *clathrin.* After LDL binding, clathrin promotes invagination of the pits and pinching off of vesicles (*"coated vesicles"*). The clathrin then dissociates off and is reused. After fusion of the vesicle with lysosomes, the LDL particles are broken down (see p. 234), and cholesterol and other lipids are used by the cells.

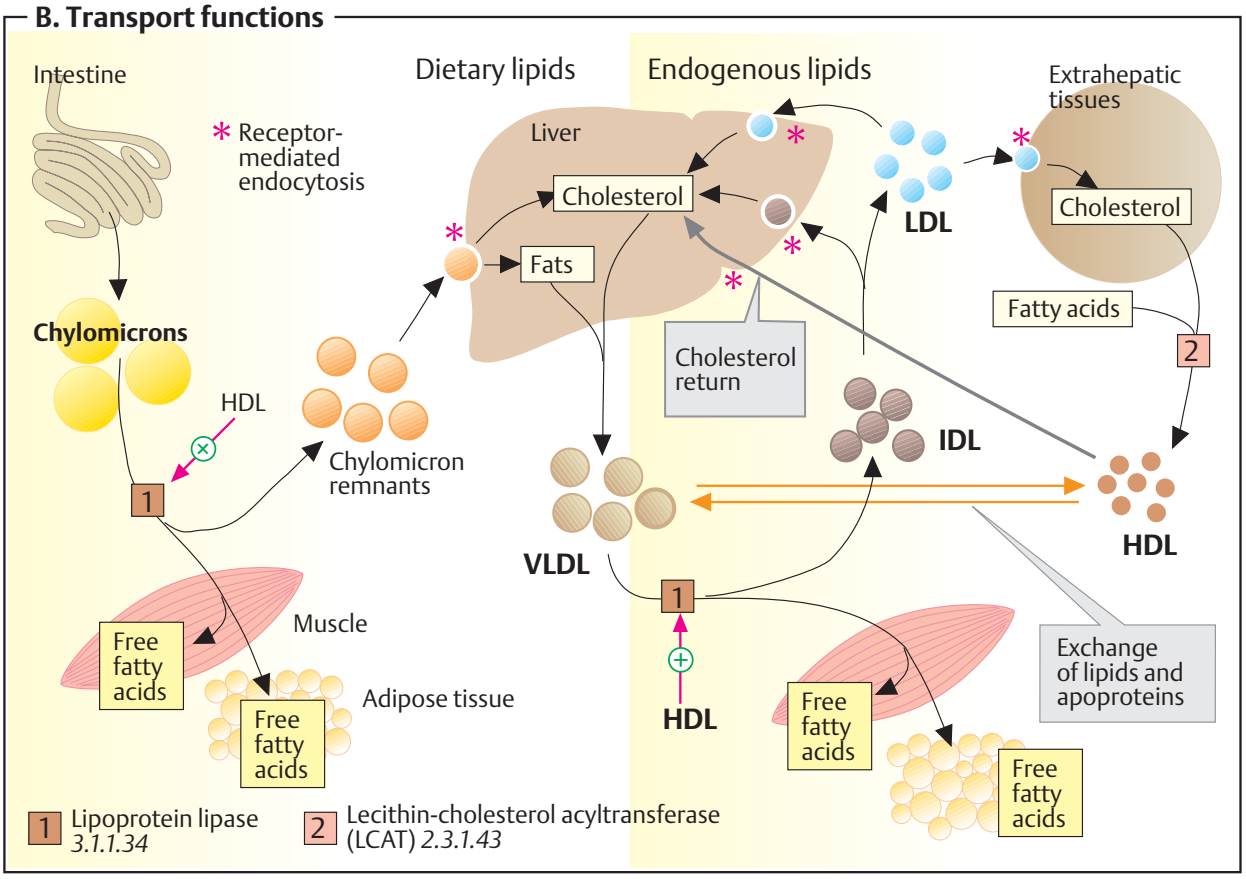
The **HDLs** also originate in the liver. They return the excess cholesterol formed in the tissues to the liver. While it is being transported, cholesterol is acylated by *lecithin cholesterol acyltransferase* (LCAT). The cholesterol esters formed are no longer amphipathic and can be transported in the core of the lipoproteins. In addition, HDLs promote chylomicron and VLDL turnover by exchanging lipids and apoproteins with them (see above).

A. Composition of lipoprotein complexes



Lipoprotein	Density ($g \cdot cm^{-3}$)
Chylomicrons	<0.95
VLDL	0.950 – 1.006
IDL	1.006 – 1.019
LDL	1.019 – 1.063
HDL	1.063 – 1.210

B. Transport functions



Hemoglobin

The most important task of the red blood cells (erythrocytes) is to **transport** molecular oxygen ($\mathbf{O_2}$) from the lungs into the tissues, and carbon dioxide ($\mathbf{CO_2}$) from the tissues back into the lungs. To achieve this, the higher organisms require a special transport system, since O_2 is *poorly soluble in water.* For example, only around 3.2 mL O_2 is soluble in 1 L blood plasma. By contrast, the protein **hemoglobin** (Hb), contained in the erythrocytes, can bind a maximum of 220 mL O_2 per liter—70 times the physically soluble amount.

The Hb content of blood, at 140–180 g L^{-1} in men and 120–160 g L^{-1} in women, is twice as high as that of the plasma proteins (50–80 g L^{-1}). Hb is therefore also responsible for the majority of the blood proteins' pH buffer capacity (see p. 288).

A. Hemoglobin: structure ◑

In adults, hemoglobin (**HbA**; see below) is a *heterotetramer* consisting of two α-chains and two β-chains, each with masses of 16 kDa. The α- and β-chains have different sequences, but are similarly folded. Some 80% of the amino acid residues form *α-helices,* which are identified using the letters A–H.

Each subunit carries a **heme group** (formula on p. 106), with a central bivalent **iron ion**. When O_2 binds to the heme iron (**Oxygenation** of Hb) and when O_2 is released (**Deoxygenation**), the oxidation stage of the iron does *not* change. Oxidation of Fe^{2+} to Fe^{3+} only occurs occasionally. The oxidized form, *methemoglobin,* is then no longer able to bind O_2. The proportion of Met-Hb is kept low by reduction (see p. 284) and usually amounts to only 1–2%.

Four of the six coordination sites of the iron in hemoglobin are occupied by the nitrogen atoms of the pyrrol rings, and another is occupied by a histidine residue of the globin (the *proximal histidine*). The iron's sixth site is coordinated with oxygen in oxyhemoglobin and with H_2O in deoxyhemoglobin.

B. Hemoglobin: allosteric effects ◑

Like aspartate carbamoyltransferase (see p. 116), Hb can exist in two different states (*conformations*), known as the T form and the R form. The **T form** (for *tense;* left) and has a much *lower O_2* af nity than the R form (for *relaxed;* right).

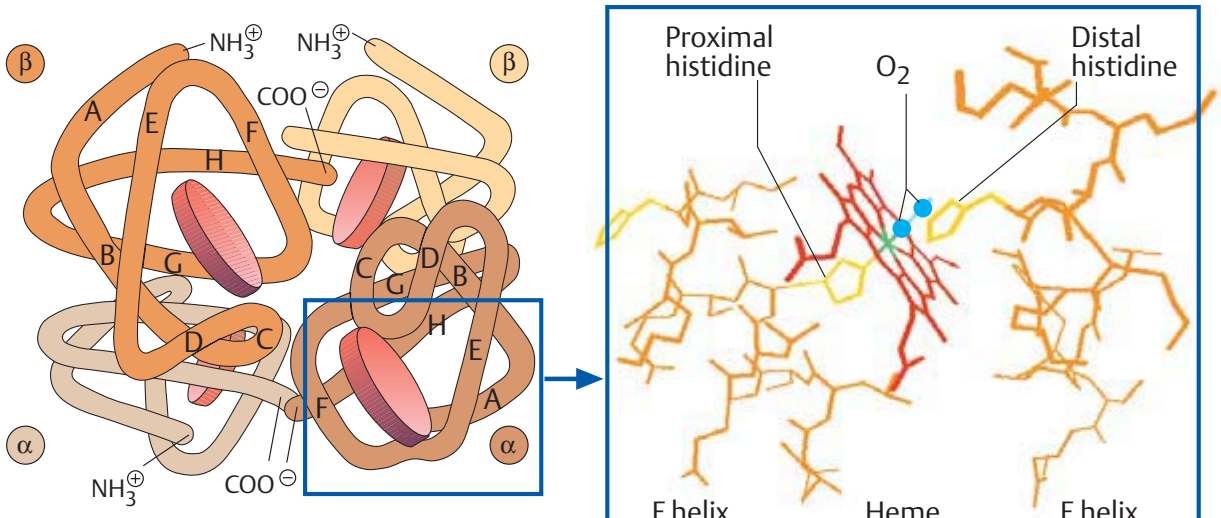
Binding of O_2 to one of the subunits of the T form leads to a local conformational change that weakens the association between the subunits. Increasing O_2 partial pressure thus means that more and more molecules convert to the higher–af nity R form. This **cooperative interaction** between the subunits increases the O_2 af nity of Hb with increasing O_2 concentrations—i. e., the O_2 saturation curve is **sigmoidal** (see p. 282).

Various **allosteric effectors** influence the equilibrium between the T and R forms and thereby regulate the O_2 binding behavior of hemoglobin (yellow arrows). The most important effectors are *CO_2, H^+,* and *2,3-bisphosphoglycerate* (see p. 282).

Further information

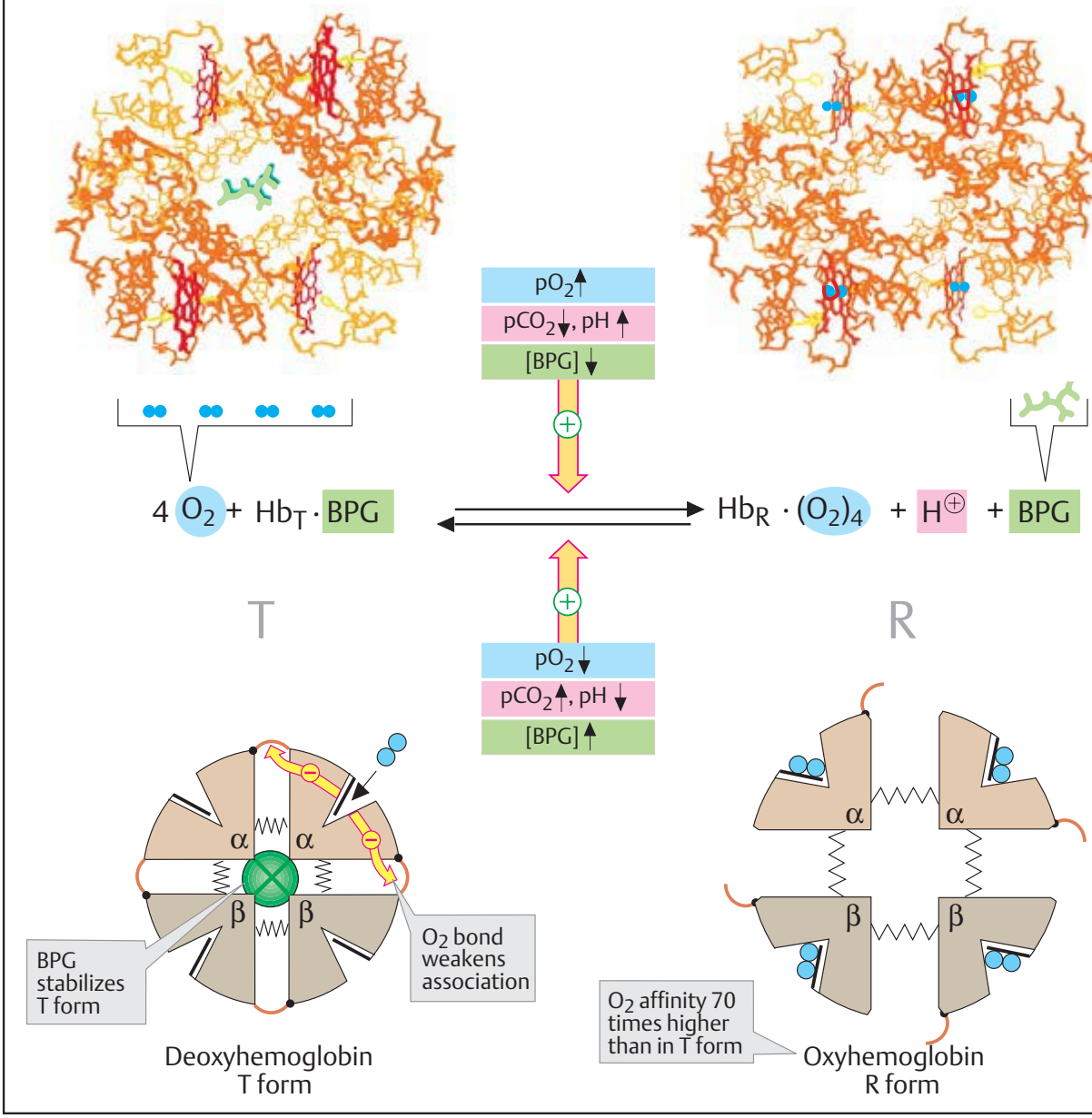
As mentioned above, hemoglobin in adults consists of two α- and two β-chains. In addition to this main form (**HbA_1**, $\alpha_2\beta_2$), adult blood also contains small amounts of a second form with a higher O_2 af nity in which the β-chains are replaced by δ-chains (**HbA_2**, $\alpha_2\delta_2$). Two other forms occur during embryonic and fetal development. In the first three months, **embryonic hemoglobins** are formed, with the structure $\zeta_2\varepsilon_2$ and $\alpha_2\varepsilon_2$. Up to the time of birth, **fetal hemoglobin** then predominates (HbF, $\alpha_2\gamma_2$), and it is gradually replaced by HbA during the first few months of life. Embryonic and fetal hemoglobins have higher O_2 af nities than HbA, as they have to take up oxygen from the maternal circulation.

A. Hemoglobin: structure



Hemoglobin A $(\alpha_2 \beta_2)$ M: 65 kDa

B. Hemoglobin: allosteric effects



Gas transport

Most tissues are constantly dependent on a supply of molecular oxygen (O_2) to maintain their oxidative metabolism. Due to its poor solubility, O_2 is bound to hemoglobin for transport in the blood (see p. 280). This not only increases the oxygen transport capacity, but also allows regulation of O_2 uptake in the lungs and O_2 release into tissues.

A. Regulation of O_2 transport ◑

When an enzyme reacts to effectors (substrates, activators, or inhibitors) with conformational changes that increase or reduce its activity, it is said to show *allosteric behavior* (see p. 116). Allosteric enzymes are usually oligomers with several subunits that mutually influence each other.

Although **hemoglobin** is not an enzyme (it releases the bound oxygen without changing it), it has all the characteristics of an allosteric protein. Its effectors include oxygen, which as a *positive homotropic effector* promotes its own binding. The **O_2 saturation curve** of hemoglobin is therefore markedly sigmoidal in shape (**2**, curve 2). The non-sigmoidal saturation curve of the muscular protein **myoglobin** is shown for comparison (curve 1). The structure of myoglobin (see p. 336) is similar to that of a subunit of hemoglobin, but as a monomer it does not exhibit any allosteric behavior.

CO_2, H^+, and a special metabolite of erythrocytes—**2,3-bisphosphoglycerate (BPG)**—act as *heterotropic effectors* of hemoglobin. BPG is synthesized from *1,3–bisphosphoglycerate,* an intermediate of glycolysis (see p. 150), and it can be returned to glycolysis again by breakdown into 2–phosphoglycerate (**1**), with loss of an ATP.

BPG binds selectively to *deoxy–Hb,* thereby increasing its amount of equilibrium. The result is *increased O_2 release* at constant pO_2. In the diagram, this corresponds to a **right shift** of the saturation curve (**2**, curve 3). CO_2 and H^+ act in the same direction as BPG. Their influence on the position of the curve has long been known as the **Bohr effect**.

The effects of CO_2 and BPG are *additive.* In the presence of both effectors, the saturation curve of isolated Hb is similar to that of whole blood (curve 4).

B. Hemoglobin and CO_2 transport ◑

Hemoglobin is also decisively involved in the transport of carbon dioxide (CO_2) from the tissues to the lungs.

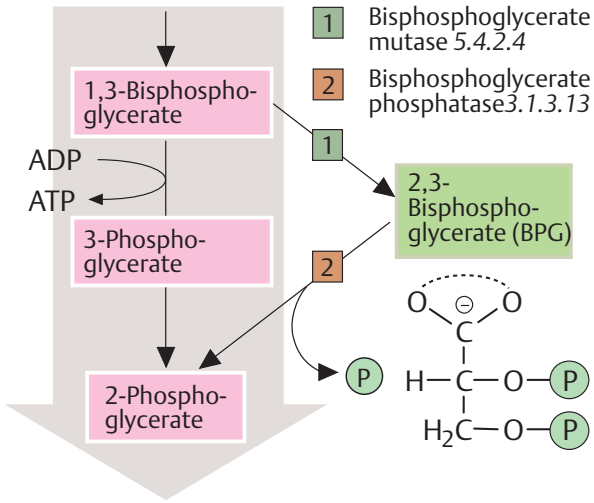
Some 5% of the CO_2 arising in the tissues is covalently bound to the N terminus of hemoglobin and transported as *carbaminohemoglobin* (not shown). About 90% of the CO_2 is first converted in the periphery into *hydrogen carbonate* (HCO_3^-), which is more soluble (bottom). In the lungs (top), CO_2 is regenerated again from HCO_3^- and can then be exhaled.

These two processes are coupled to the oxygenation and deoxygenation of Hb. *Deoxy–Hb* is a stronger base than oxy–Hb. It therefore binds additional protons (about 0.7 H^+ per tetramer), which promotes the formation of HCO_3^- from CO_2 in the peripheral tissues. The resulting HCO_3^- is released into the plasma via an antiporter in the erythrocyte membrane in exchange for Cl^-, and passes from the plasma to the lungs. In the lungs, the reactions described above then proceed in reverse order: deoxy-Hb is oxygenated and releases protons. The protons shift the HCO_3/CO_2 equilibrium to the left and thereby promote CO_2 release.

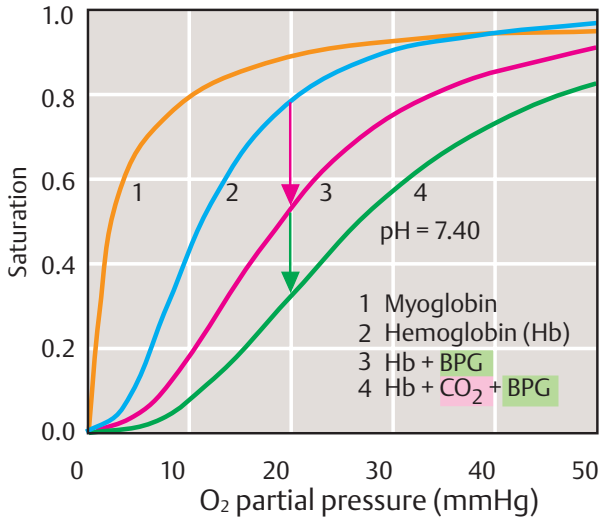
O_2 binding to Hb is regulated by H^+ ions (i.e., by the pH value) via the same mechanism. High concentrations of CO_2 such as those in tissues with intensive metabolism locally increase the H^+ concentration and thereby reduce hemoglobin's O_2 af nity (Bohr effect; see above). This leads to increased O_2 release and thus to an improved oxygen supply.

The adjustment of the equilibrium between CO_2 and HCO_3^- is relatively slow in the uncatalyzed state. It is therefore accelerated in the erythrocytes by *carbonate dehydratase* (carbonic anhydrase) [1])—an enzyme that occurs in high concentrations in the erythrocytes.

A. Regulation of O_2 transport

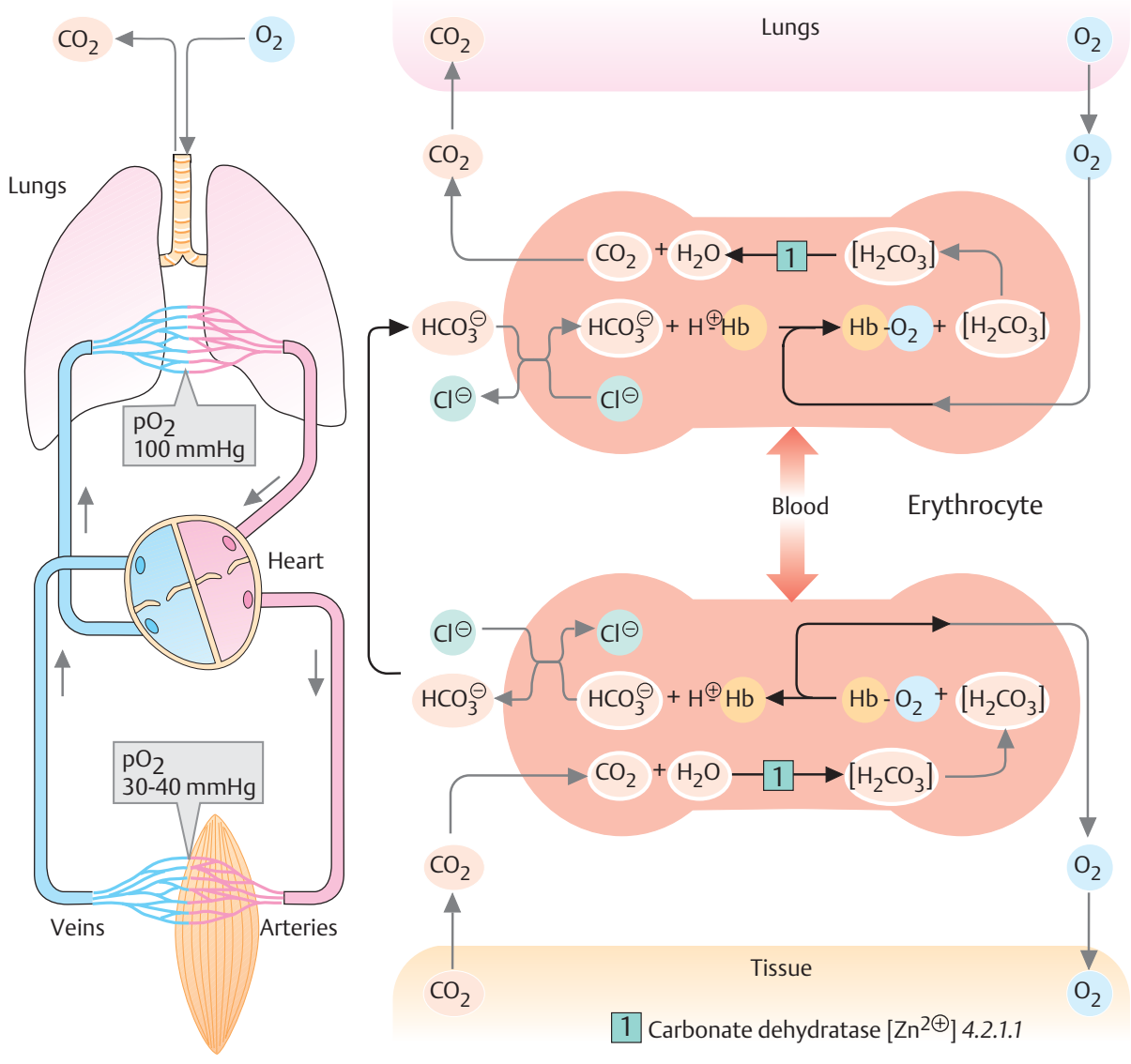


1. BPG metabolism



2. Saturation curves

D. Hemoglobin and CO_2 transport



Erythrocyte metabolism

Cells living in aerobic conditions are dependent on molecular oxygen for energy production. On the other hand, O_2 constantly gives rise to small quantities of toxic substances known as **reactive oxygen species** (ROS). These substances are powerful *oxidation agents* or extremely reactive *free radicals* (see p. 32), which damage cellular structures and functional molecules. Due to their role in O_2 transport, the erythrocytes are constantly exposed to high concentrations of O_2 and are therefore particularly at risk from ROS.

A. Reactive oxygen species ◑

The dioxygen molecule (O_2) contains two unpaired electrons—i. e., it is a *diradical.* Despite this, O_2 is relatively stable due to its special electron arrangement. However, if the molecule takes up an extra electron (**a**), the highly reactive **superoxide radical** (O_2^-) arises. Another reduction step (**b**) leads to the **peroxide anion** (O_2^{2-}), which easily binds protons and thus becomes **hydrogen peroxide** (H_2O_2). Inclusion of a third electron (**c**) leads to cleavage of the molecule into the ions O^{2-} and O^-. While O^{2-} can form **water** by taking up two protons, protonation of O^- provides the extremely dangerous **hydroxy radical** (OH). A fourth electron transfer and subsequent protonation also convert O^- into water.

The synthesis of ROS can be catalyzed by iron ions, for example. Reaction of O_2 with FMN or FAD (see p. 32) also constantly produces ROS. By contrast, reduction of O_2 by *cytochrome c-oxidase* (see p. 140) is "clean," as the enzyme does not release the intermediates. In addition to antioxidants (**B**), **enzymes** also provide protection against ROS: *superoxide dismutase* [1] breaks down ("disproportionates") two superoxide molecules into O_2 and the less damaging H_2O_2. The latter is in turn disproportionated into O_2 and H_2O by heme-containing *catalase* [2].

B. Biological antioxidants ◑

To protect them against ROS and other radicals, all cells contain **antioxidants**. These are *reducing agents* that react easily with oxidative substances and thus protect more important molecules from oxidation. Biological antioxidants include vitamins C and E (see pp. 364, 368), coenzyme Q (see p. 104), and several carotenoids (see pp. 132, 364). Bilirubin, which is formed during heme degradation (see p. 194), also serves for protection against oxidation.

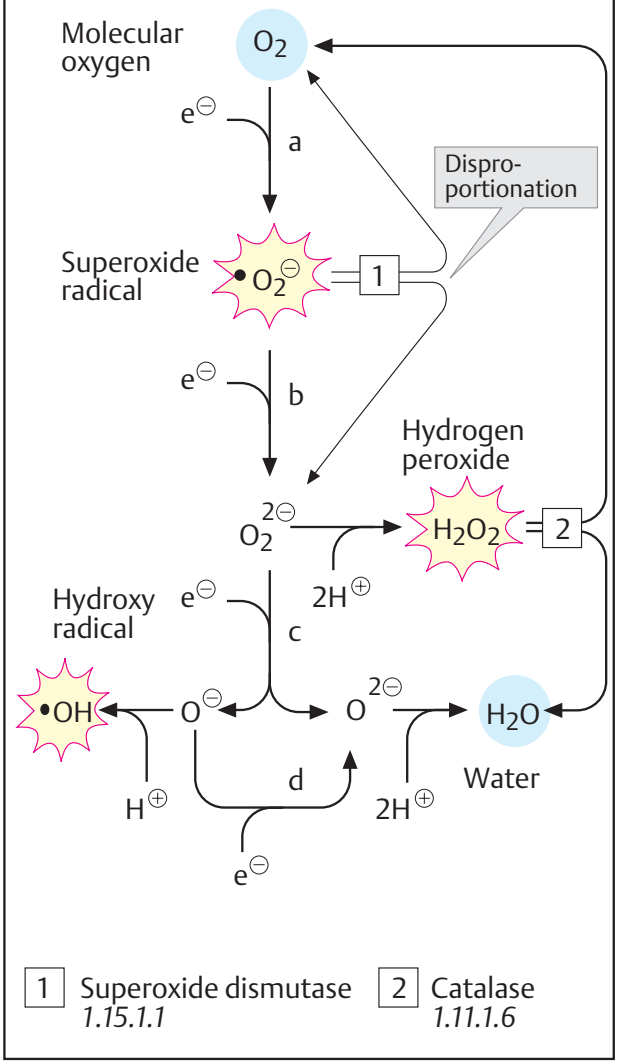
Glutathione, a tripeptide that occurs in high concentrations in almost all cells, is particularly important. Glutathione (sequence: Glu–Cys–Gly) contains an atypical γ-peptide bond between Glu and Cys. The thiol group of the cysteine residue is redox-active. Two molecules of the reduced form (GSH, top) are bound to the disulfide (GSSG, bottom) during oxidation.

C. Erythrocyte metabolism ◑

Erythrocytes also have systems that can inactivate ROS (superoxide dismutase, catalase, GSH). They are also able to repair damage caused by ROS. This requires products that are supplied by the erythrocytes' **maintenance metabolism**, which basically only involves anaerobic glycolysis (see p. 150) and the pentose phosphate pathway (PPP; see p. 152).

The **ATP** formed during glycolysis serves mainly to supply *Na^+/K^+-ATPase,* which maintains the erythrocytes' membrane potential. The allosteric effector **2,3-BPG** (see p. 282) is also derived from glycolysis. The PPP supplies **$NADPH+H^+$**, which is needed to regenerate **glutathione** (GSH) from GSSG with the help of *glutathione reductase* [3]. GSH, the most important antioxidant in the erythrocytes, serves as a coenzyme for *glutathione peroxidase* [5]. This selenium-containing enzyme detoxifies H_2O_2 and hydroperoxides, which arise during the reaction of ROS with unsaturated fatty acids in the erythrocyte membrane. The reduction of methemoglobin ($Hb\,Fe^{3+}$) to Hb ($Hb\,Fe^{2+}$, [4]) is carried out by GSH or ascorbate by a non-enzymatic pathway; however, there are also NAD(P)H-dependent Met-Hb reductases.

A. Reactive oxygen species



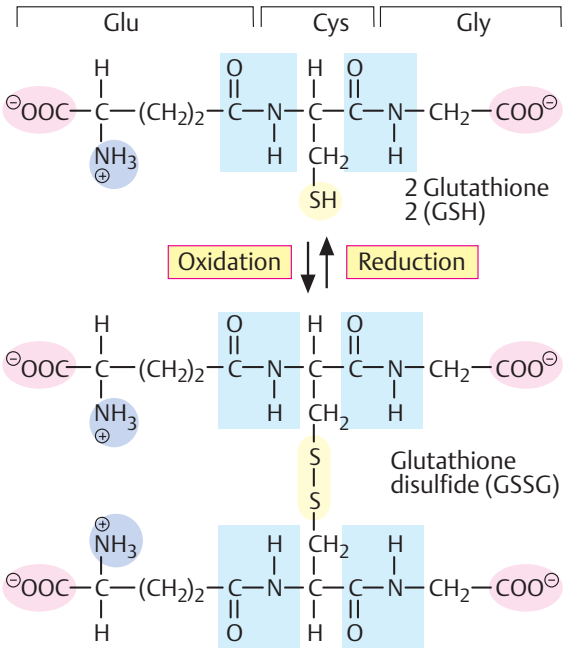
1 Superoxide dismutase *1.15.1.1*

2 Catalase *1.11.1.6*

B. Biological antioxidants

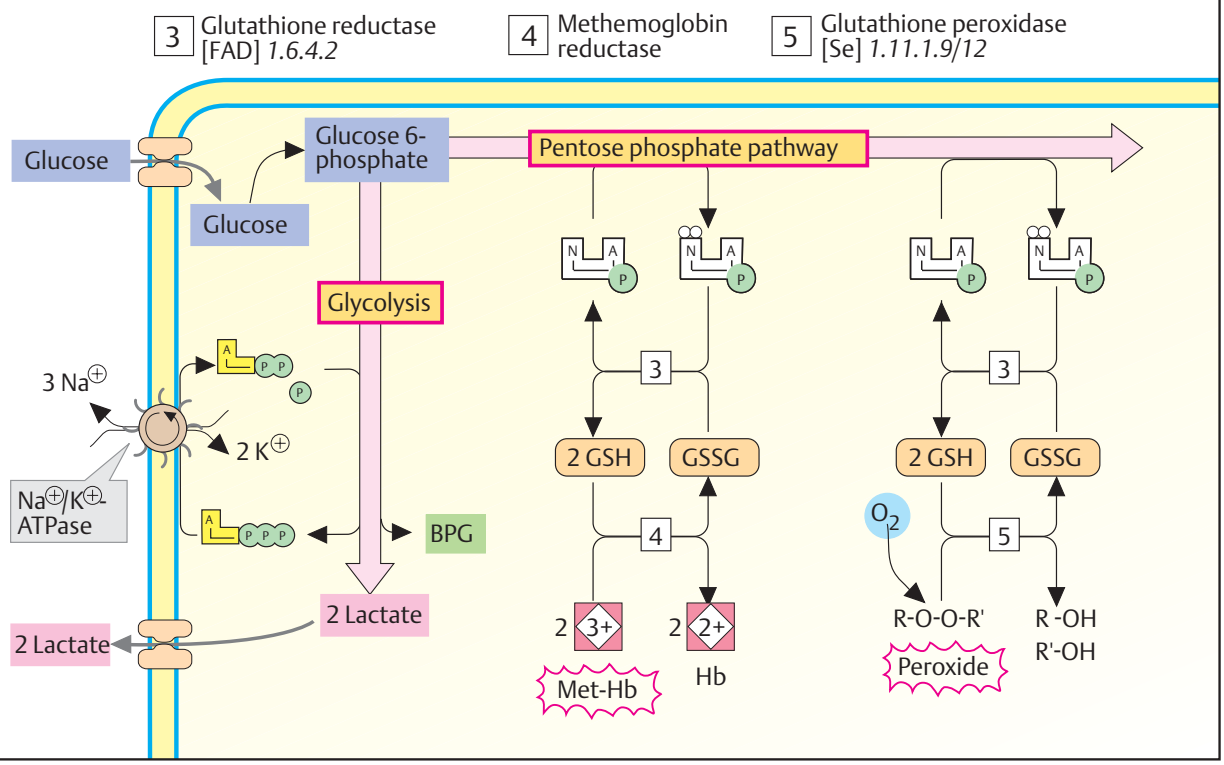
Quinols and enols	α-Tocopherol (vitamin E) Ubiquinol (coenzyme Q) Ascorbic acid (vitamin C)
Carotenoids	β-Carotin Lycopin
Others	Glutathione Bilirubin

1. Examples



2. Glutathione

C. Erythrocyte metabolism



Iron metabolism

A. Distribution of iron ◑

Iron (Fe) is quantitatively the most important trace element (see p. 362). The human body contains 4–5 g iron, which is almost exclusively present in protein-bound form. Approximately three-quarters of the total amount is found in **heme proteins** (see pp. 106, 192), mainly hemoglobin and myoglobin. About 1% of the iron is bound in **iron–sulfur clusters** (see p. 106), which function as cofactors in the respiratory chain, in photosynthesis, and in other redox chains. The remainder consists of iron in transport and storage proteins (transferrin, ferritin; see **B**).

B. Iron metabolism ◑

Iron can only be resorbed by the bowel in bivalent form (i. e., as Fe^{2+}). For this reason, reducing agents in food such as ascorbate (vitamin C; see p. 368) promote **iron uptake**. Via transporters on the luminal and basal side of the enterocytes, Fe^{2+} enters the blood, where it is bound by *transferrin*. Part of the iron that is taken up is stored in the bowel in the form of *ferritin* (see below). Heme groups can also be resorbed by the small intestine.

Most of the resorbed iron serves for the formation of red blood cells in the bone marrow (**erythropoiesis**, top). As discussed on p. 192, it is only in the final step of hem biosynthesis that Fe^{2+} is incorporated by *ferrochelatase* into the previously prepared tetrapyrrol framework.

In the blood, 2.5–3.0 g of hemoglobin iron circulates as a component of the erythrocytes (top right). Over the course of several months, the flexibility of the red blood cells constantly declines due to damage to the membrane and cytoskeleton. Old erythrocytes of this type are taken up by macrophages in the spleen and other organs and broken down. The organic part of the heme is oxidized into bilirubin (see p. 194), while the iron returns to the plasma pool. The quantity of heme iron recycled per day is much larger than the amount resorbed by the intestines.

Transferrin, a β-globulin with a mass of 80 kDa, serves to transport iron in the blood. This monomeric protein consists of two similar domains, each of which binds an Fe^{2+} ion very tightly. Similar iron transport proteins are found in secretions such as saliva, tears, and milk; these are known as lactoferrins (bottom right). Transferrin and the lactoferrins maintain the concentration of *free* iron in body fluids at values below 10^{-10} mol L^{-1}. This low level prevents bacteria that require free iron as an essential growth factor from proliferating in the body. Like LDLs (see p. 278), transferrin and the lactoferrins are taken up into cells by *receptor-mediated endocytosis*.

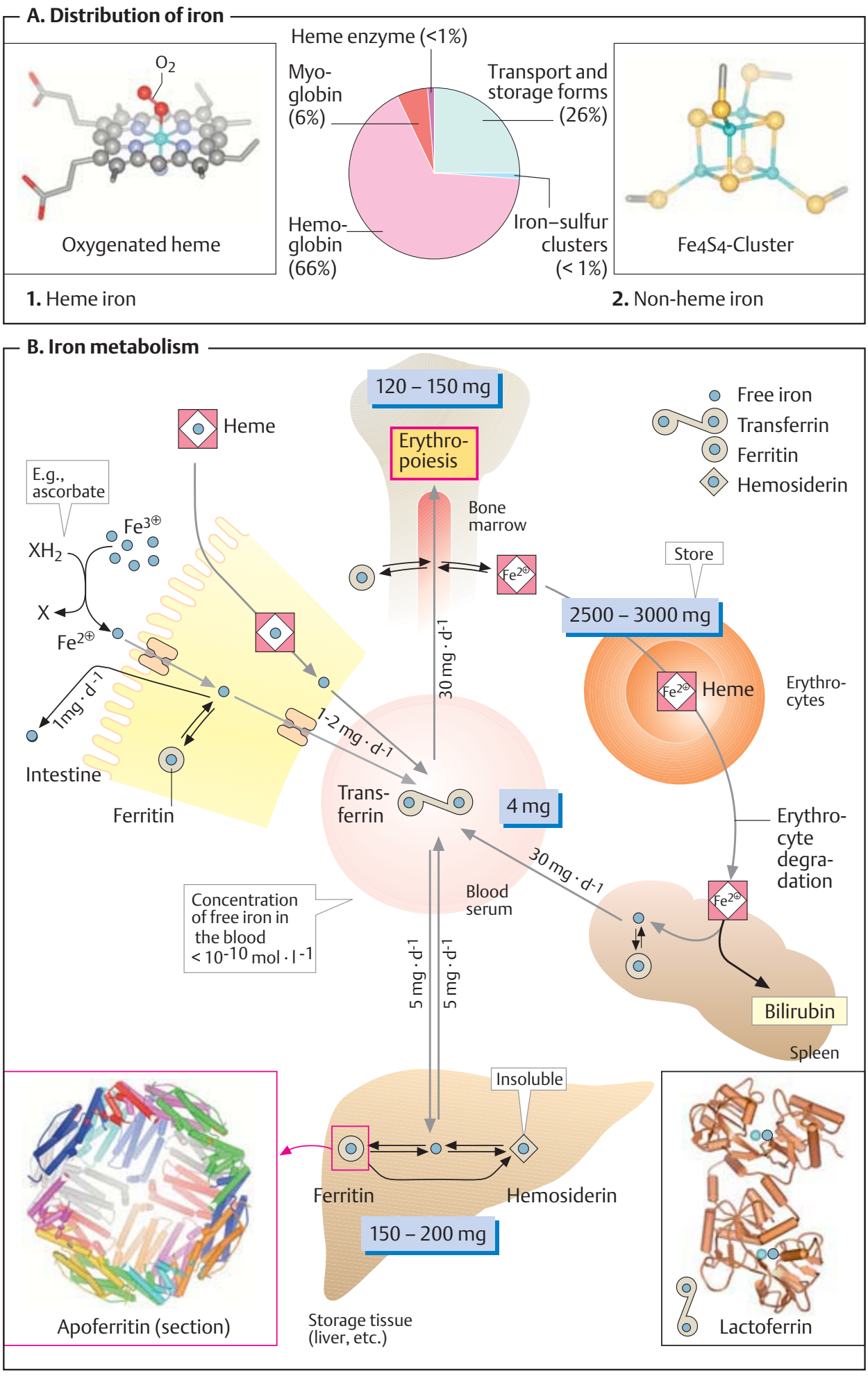
Excess iron is incorporated into **ferritin** and stored in this form in the liver and other organs. The ferritin molecule consists of 24 subunits and has the shape of a hollow sphere (bottom left). It takes up Fe^{2+} ions, which in the process are oxidized to Fe^{3+} and then deposited in the interior of the sphere as *ferrihydrate*. Each ferritin molecule is capable of storing several thousand iron ions in this way. In addition to ferritin, there is another storage form, **hemosiderin**, the function of which is not yet clear.

Further information

Disturbances of the iron metabolism are frequent and can lead to severe disease pictures.

Iron deficiency is usually due to blood loss, or more rarely to inadequate iron uptake. During pregnancy, increased demand can also cause iron deficiency states. In severe cases, reduced hemoglobin synthesis can lead to **anemia** ("iron-deficiency anemia"). In these patients, the erythrocytes are smaller and have less hemoglobin. As their membrane is also altered, they are prematurely eliminated in the spleen.

Disturbances resulting from raised iron concentrations are less frequent. Known as **hemochromatoses**, these conditions can have genetic causes, or may be due to repeated administration of blood transfusions. As the body has practically no means of excreting iron, more and more stored iron is deposited in the organs over time in patients with untreated hemochromatosis, ultimately leading to severe disturbances of organ function.



Acid–base balance

A. Hydrogen ion concentration in the blood plasma ◑

The H^+ concentration in the blood and extracellular space is approximately 40 nM ($4 \cdot 10^{-8}$ mol L^{-1}). This corresponds to a pH of 7.40. The body tries to keep this value constant, as large shifts in pH are incompatible with life.

The pH value is kept constant by **buffer systems** that cushion minor disturbances in the *acid–base balance* (**C**). In the longer term, the decisive aspect is maintaining a balanced equilibrium between H^+ production and uptake and H^+ release. If the blood's buffering capacity is not suf cient, or if the acid–base balance is not in equilibrium—e. g., in kidney disease or during *hypoventilation* or *hyperventilation*—shifts in the plasma pH value can occur. A reduction by more than 0.03 units is known as **acidosis,** and an increase is called **alkalosis.**

B. Acid–base balance ◑

Protons are mainly derived from two sources—free acids in the diet and sulfur–containing amino acids. *Acids* taken up with food—e. g., citric acid, ascorbic acid, and phosphoric acid—already release protons in the alkaline pH of the intestinal tract. More important for proton balance, however, are the amino acids **methionine** and **cysteine**, which arise from protein degradation in the cells. Their S atoms are oxidized in the liver to form sulfuric acid, which supplies protons by dissociation into sulfate.

During anaerobic glycolysis in the muscles and erythrocytes, glucose is converted into **lactate**, releasing protons in the process (see p. 338). The synthesis of the **ketone bodies** acetoacetic acid and 3–hydroxybutyric acid in the liver (see p. 312) also releases protons. Normally, the amounts formed are small and of little influence on the proton balance. If acids are formed in large amounts, however (e. g., during *starvation* or in *diabetes mellitus;* see p. 160), they strain the buffer systems and can lead to a reduction in pH (**metabolic acidoses**; *lactacidosis* or *ketoacidosis*).

Only the **kidney** is capable of excreting protons in exchange for Na^+ ions (see p. 326). In the urine, the H^+ ions are buffered by NH_3 and phosphate.

C. Buffer systems in the plasma ◑

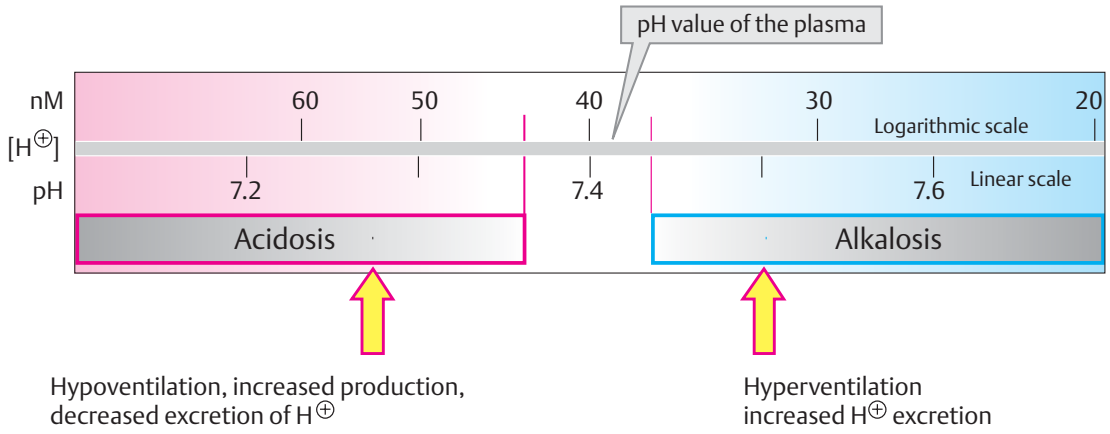
The **buffering capacity** of a buffer system depends on its concentration and its pK_a value. The strongest effect is achieved if the pH value corresponds to the buffer system's pK_a value (see p. 30). For this reason, weak acids with pK_a values of around 7 are best suited for buffering purposes in the blood.

The most important buffer in the blood is the **CO_2/bicarbonate buffer**. This consists of water, carbon dioxide (CO_2, the anhydride of carbonic acid H_2CO_3), and hydrogen carbonate (HCO_3^-, bicarbonate). The adjustment of the balance between CO_2 and HCO_3^- is accelerated by the zinc-containing enzyme *carbonate dehydratase* (carbonic anhydrase [1]; see also p. 282). At the pH value of the plasma, HCO_3^- and CO_2 are present in a ratio of about 20 : 1. However, the CO_2 in solution in the blood is in equilibrium with the gaseous CO_2 in the pulmonary alveoli. The CO_2/HCO_3^- system is therefore a powerful *open buffer system,* despite having a not entirely optimal pK_a value of 6.1. Faster or slower respiration increases or reduces CO_2 release in the lungs. This shifts the CO_2/HCO_3^- ratio and thus the plasma pH value (respiratory acidosis or alkalosis). In this way, respiration can compensate to a certain extent for changes in plasma pH values. However, it does *not* lead to the excretion of protons.

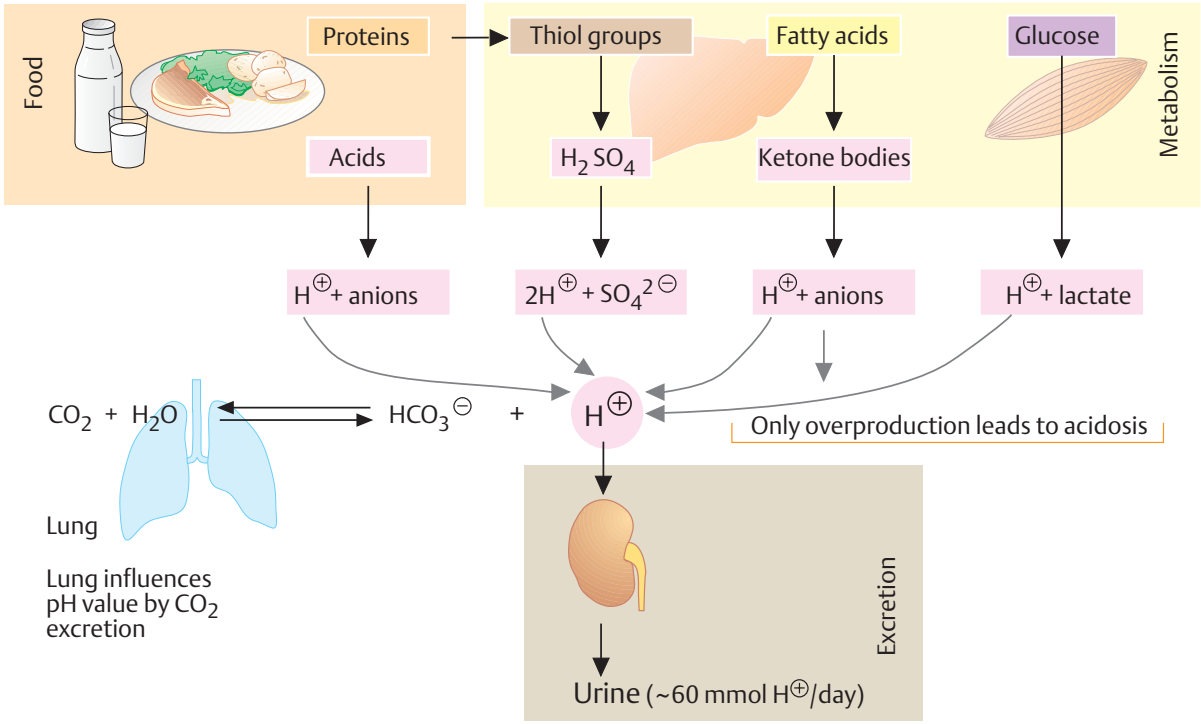
Due to their high concentration, **plasma proteins**—and **hemoglobin** in the erythrocytes in particular—provide about one-quarter of the blood's buffering capacity. The buffering effect of proteins involves contributions from all of the ionizable side chains. At the pH value of blood, the acidic amino acids (Asp, Glu) and histidine are particularly effective.

The second dissociation step in **phosphate** (H_2PO_4/HPO_4^{2-}) also contributes to the buffering capacity of the blood plasma. Although the pK_a value of this system is nearly optimal, its contribution remains small due to the low total concentration of phosphate in the blood (around 1 mM).

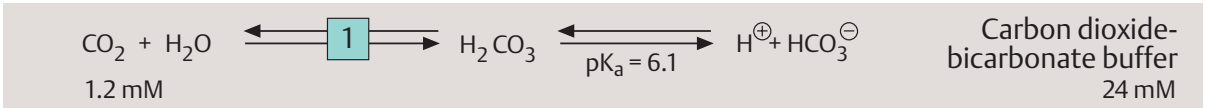
A. Hydrogen ion concentration in the blood plasma

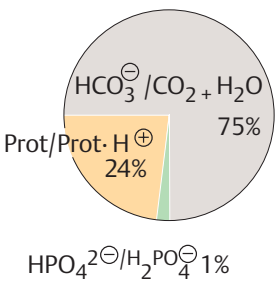


B. Acid–base balance

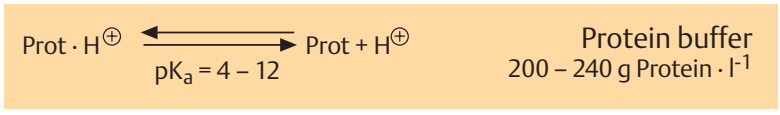


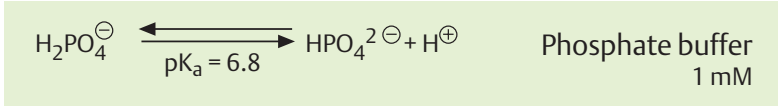
C. Buffer systems in the plasma





Buffering capacity





1 Carbonate dehydratase *4.2.1.1*

Blood clotting

Following injury to blood vessels, **hemostasis** ensures that blood loss is minimized. Initially, thrombocyte activation leads to contraction of the injured vessel and the formation of a loose clot consisting of thrombocytes (**hemostasis**). Slightly later, the action of the enzyme *thrombin* leads to the formation and deposition in the thrombus of polymeric fibrin (**coagulation, blood clotting**). The coagulation process is discussed here in detail.

A. Blood clotting ◑

The most important reaction in blood clotting is the conversion, catalyzed by *thrombin,* of the soluble plasma protein *fibrinogen* (factor I) into polymeric *fibrin,* which is deposited as a fibrous network in the primary thrombus. Thrombin (factor IIa) is a serine proteinase (see p. 176) that cleaves small peptides from fibrinogen. This exposes binding sites that spontaneously allow the fibrin molecules to aggregate into polymers. Subsequent covalent cross-linking of fibrin by a *transglutaminase* (factor XIII) further stabilizes the thrombus.

Normally, thrombin is present in the blood as an inactive proenzyme (see p. 270). Prothrombin is activated in two different ways, both of which represent cascades of enzymatic reactions in which inactive proenzymes (zymogens, symbol: circle) are proteolytically converted into active **proteinases** (symbol: sector of a circle). The proteinases activate the next proenzyme in turn, and so on. Several steps in the cascade require additional **protein factors** (factors III, Va and VIIIa) as well as anionic **phospholipids** (PL; see below) and **Ca^{2+}** ions. Both pathways are activated by injuries to the vessel wall.

In the **extravascular pathway** (right), *tissue thromboplastin* (factor III), a membrane protein in the deeper layers of the vascular wall, activates coagulation factor VII. The activated form of this (VIIa) autocatalytically promotes its own synthesis and also generates the active factors IXa and Xa from their precursors. With the aid of factor VIIIa, PL, and Ca^{2+}, factor IXa produces additional Xa, which finally—with the support of Va, PL, and Ca^{2+}—releases active thrombin.

The **intravascular pathway** (left) is probably also triggered by vascular injuries. It leads in five steps via factors XIIa, XIa, IXa, and Xa to the activation of prothrombin. The significance of this pathway in vivo has been controversial since it was found that a genetic deficiency in factor XII does not lead to coagulation disturbances.

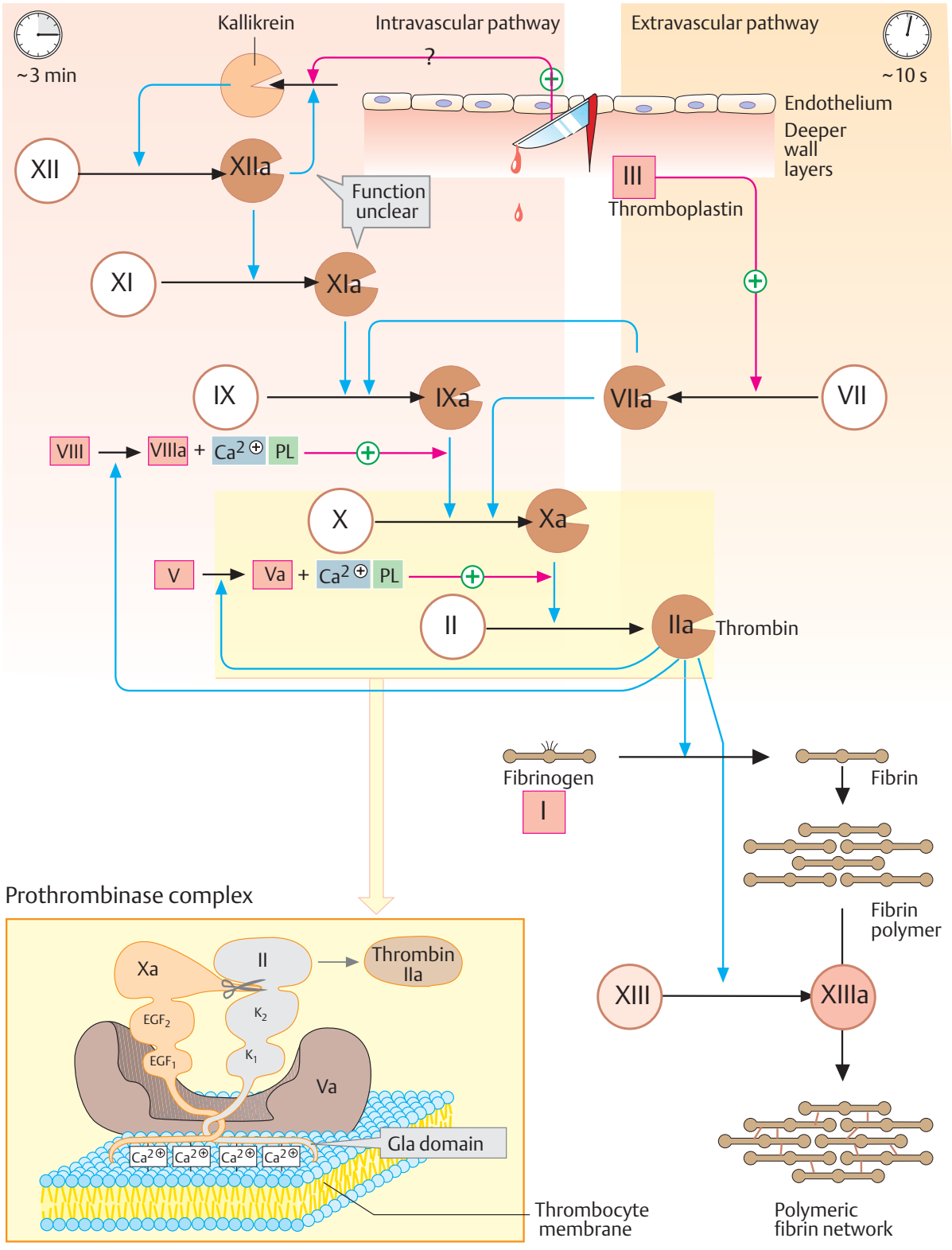
Both pathways depend on the presence of activated **thrombocytes**, on the surface of which several reactions take place. For example, the *prothrombinase complex* (left) forms when factors Xa and II, with the help of Va, bind via Ca^{2+} ions to anionic phospholipids in the thrombocyte membrane. For this to happen, factors II and X have to contain the non-proteinogenic amino acid **γ-carboxyglutamate** (Gla; see p. 62), which is formed in the liver by post-translational carboxylation of the factors. The Gla residues are found in groups in special domains that create contacts to the Ca^{2+} ions. Factors VII and IX are also linked to membrane phospholipids via Gla residues.

Substances that bind Ca^{2+} ions (e. g., *citrate*) prevent Gla-containing factors from attaching to the membrane and therefore inhibit coagulation. Antagonists of *vitamin K,* which is needed for synthesis of the Gla residues (see p. 364) also have anticoagulatory effects. These include dicumarol, for example.

Active **thrombin** not only converts fibrinogen into fibrin, but also indirectly promotes its own synthesis by catalyzing the activation of factors V and VIII. In addition, it catalyzes the activation of factor XIII and thereby triggers the cross-linking of the fibrin.

Regulation of blood clotting (not shown). To prevent the coagulation reaction from becoming excessive, the blood contains a number of anticoagulant substances, including highly effective proteinase inhibitors. For example, *antithrombin III* binds to various serine proteinases in the cascade and thereby inactivates them. *Heparin,* an anticoagulant glycosaminoglycan (see p. 346), potentiates the effect of antithrombin III. *Thrombomodulin,* which is located on the vascular endothelia, also inactivates thrombin. A glycoprotein known as *Protein C* ensures proteolytic degradation of factors V and VIII. As it is activated by thrombin, coagulation is shut down in this way.

A. Blood clotting



Coagulation factors

- I Fibrinogen
- ◆ II Prothrombin* *3.4.21.5*
- III Tissue factor/thromboplastin
- IV $Ca^{2\oplus}$
- V Proaccelerin
- VI Synonym for Va
- ◆ VII Proconvertin* *3.4.21.21*
- VIII Antihemophilic factor A
- ◆ IX Christmas factor* *3.4.21.22*
- ◆ X Stuart–Prower factor* *3.4.21.6*
- XI Plasma thromboplastin antecedent* (PTA) *3.4.21.27*
- XII Hageman factor* *3.4.21.38*
- XIII Fibrin-stabilizing factor* *2.3.2.13*

* Proenzyme
◆ Contains γ-carboxyglutamate

Fibrinolysis, blood groups

A. Fibrinolysis ◑

The fibrin thrombus resulting from blood clotting (see p. 290) is dissolved again by *plasmin,* a serine proteinase found in the blood plasma. For this purpose, the precursor *plasminogen* first has to be proteolytically activated by enzymes from various tissues. This group includes the *plasminogen activator* from the kidney (*urokinase*) and *tissue plasminogen activator* (t-PA) from vascular endothelia. By contrast, the plasma protein α_2-antiplasmin, which binds to active plasmin and thereby inactivates it, inhibits fibrinolysis.

Urokinase, t-PA, and streptokinase, a bacterial proteinase with similar activity, are used clinically to dissolve thrombi following *heart attacks.* All of these proteins are expressed recombinantly in bacteria (see p. 262).

B. Blood groups: the ABO system ◑

During blood transfusions, immune reactions can occur that destroy the erythrocytes transfused from the donor. These reactions result from the formation of antibodies (see p. 300) directed to certain surface structures on the erythrocytes. Known as **blood group antigens**, these are *proteins* or *oligosaccharides* that can differ from individual to individual. More than 20 different blood group systems are now known. The ABO system and the Rh system are of particular clinical importance.

In the **ABO system**, the carbohydrate parts of glycoproteins or glycolipids act as antigens. In this relatively simple system, there are four *blood groups* (A, B, AB, and 0). In individuals with blood groups A and B, the antigens consist of tetrasaccharides that only differ in their terminal sugar (galactose or *N*-acetylgalactosamine). Carriers of the AB blood group have both antigens (A *and* B). Blood group 0 arises from an oligosaccharide (the H antigen) that lacks the terminal residue of antigens A and B. The molecular causes for the differences between blood groups are mutations in the *glycosyl transferases* that transfer the terminal sugar to the core oligosaccharide.

Antibodies are only formed against antigens that the individual concerned does *not* possess. For example, carriers of blood group A form antibodies against antigen B ("anti-B"), while carriers of group B form antibodies against antigen A ("anti-A"). Individuals with blood group 0 form both types, and those with blood group AB do not form any of these antibodies.

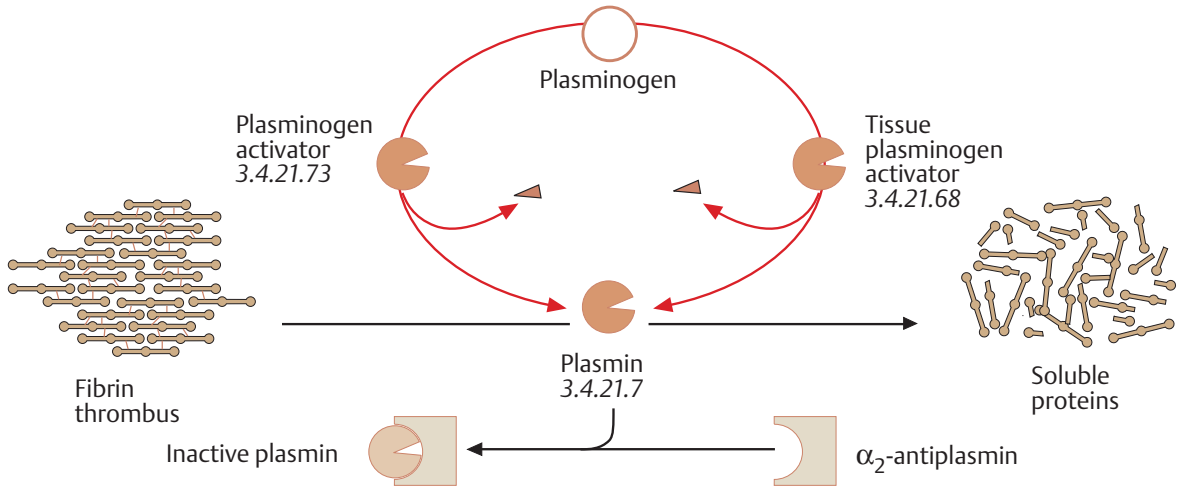
If blood from blood group A is transfused into the circulation of an individual with blood group B, for example, then the anti-A present there binds to the A antigens. The donor erythrocytes marked in this way are recognized and destroyed by the complement system (see p. 298). In the test tube, *agglutination* of the erythrocytes can be observed when donor and recipient blood are incompatible.

The recipient's serum should not contain any antibodies against the donor erythrocytes, and the donor serum should not contain any antibodies against the recipient's erythrocytes. Donor blood from blood group 0 is unproblematic, as its erythrocytes do not possess any antibodies and therefore do not react with anti-A or anti-B in the recipient's blood. Conversely, blood from the AB group can only be administered to recipients with the AB group, as these are the only ones without antibodies.

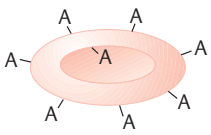
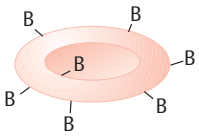
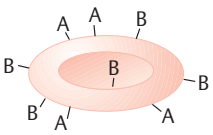
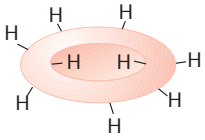
In the **Rh system** (not shown), proteins on the surface of the erythrocytes act as antigens. These are known as "rhesus factors," as the system was first discovered in rhesus monkeys.

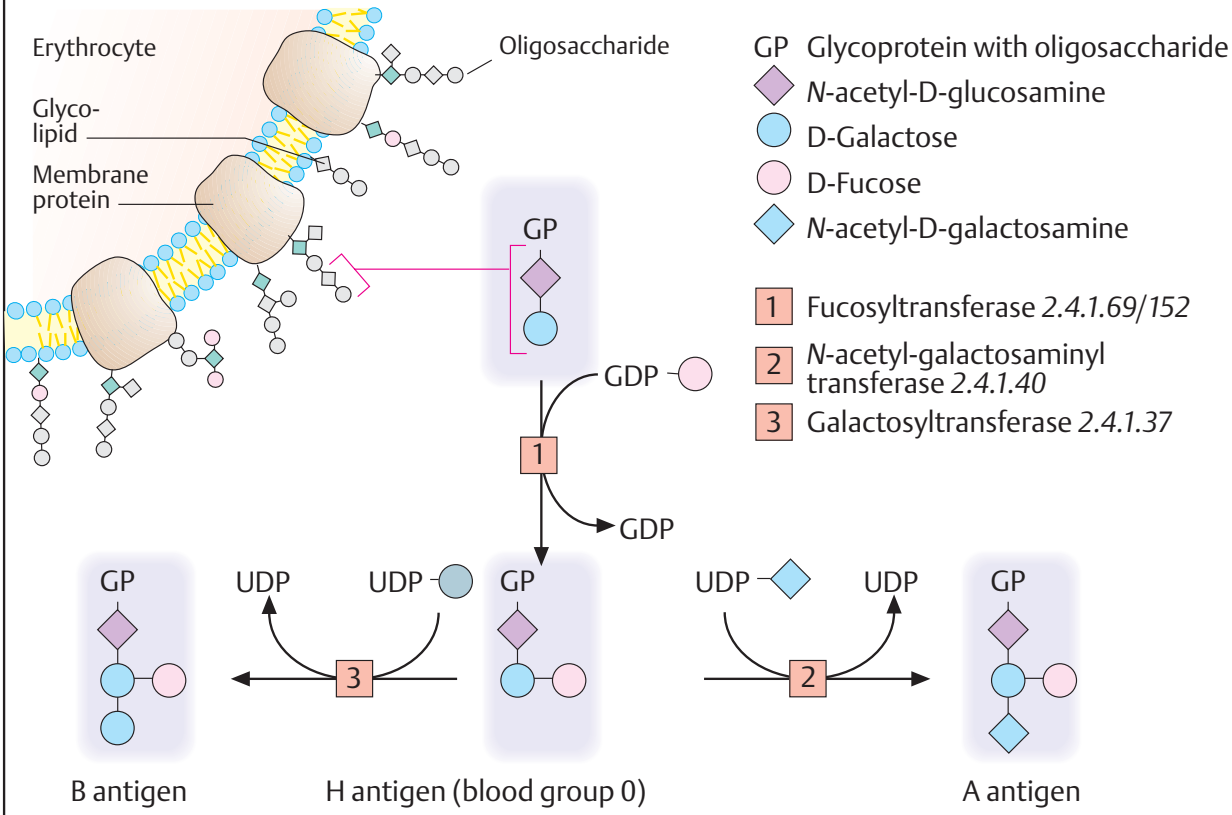
The rhesus D antigen occurs in 84% of all white individuals, who are therefore *"Rh-positive."* If an Rh-positive child is born to an Rh-negative mother, fetal erythrocytes can enter the mother's circulation during birth and lead to the formation of antibodies (IgG) against the D antigen. This initially has no acute effects on the mother or child. Complications only arise when there is a second pregnancy with an Rh-positive child, as maternal anti-D antibodies cross the placenta to the fetus even before birth and can trigger destruction of the child's Rh-positive erythrocytes (*fetal erythroblastosis*).

A. Fibrinolysis



B. Blood groups: the AB0 system

Blood group	A	B	AB	0
Genotypes	AA and A0	BB and B0	AB	00
Antigens				
Antibodies in blood	anti-B	anti-A	—	anti-A anti-B
Frequency in central Europe	40%	16%	4%	40%



Immune response

Viruses, bacteria, fungi, and parasites that enter the body of vertebrates of are recognized and attacked by the **immune system**. Endogenous cells that have undergone alterations—e. g., tumor cells—are also usually recognized as foreign and destroyed. The immune system is supported by physiological changes in infected tissue, known as **inflammation**. This reaction makes it easier for the immune cells to reach the site of infection.

Two different systems are involved in the immune response. The **innate immune system** is based on receptors that can distinguish between bacterial and viral surface structures or foreign proteins (known as *antigens*) and those that are endogenous. With the help of these receptors, *phagocytes* bind to the pathogens, absorb them by endocytosis, and break them down. The complement system (see p. 298) is also part of the innate system.

The **acquired** (adaptive) **immune system** is based on the ability of the *lymphocytes* to form highly specific antigen receptors "on suspicion," without ever having met the corresponding antigen. In humans, there are several billion different lymphocytes, each of which carries a different antigen receptor. If this type of receptor recognizes "its" cognate antigen, the lymphocyte carrying it is activated and then plays its special role in the immune response.

In addition, a distinction is made between cellular and humoral immune responses. The *T lymphocytes* (T cells) are responsible for **cellular immunity**. They are named after the thymus, in which the decisive steps in their differentiation take place. Depending on their function, another distinction is made between *cytotoxic T cells* (green) and *helper T cells* (blue). **Humoral immunity** is based on the activity of the *B lymphocytes* (B cells, light brown), which mature in the bone marrow. After activation by T cells, B cells are able to release soluble forms of their specific antigen receptors, known as *antibodies* (see p. 300), into the blood plasma. The immune system's "memory" is represented by memory cells. These are particularly long–lived cells that can arise from any of the lymphocyte types described.

A. Simplified diagram of the immune response ◑

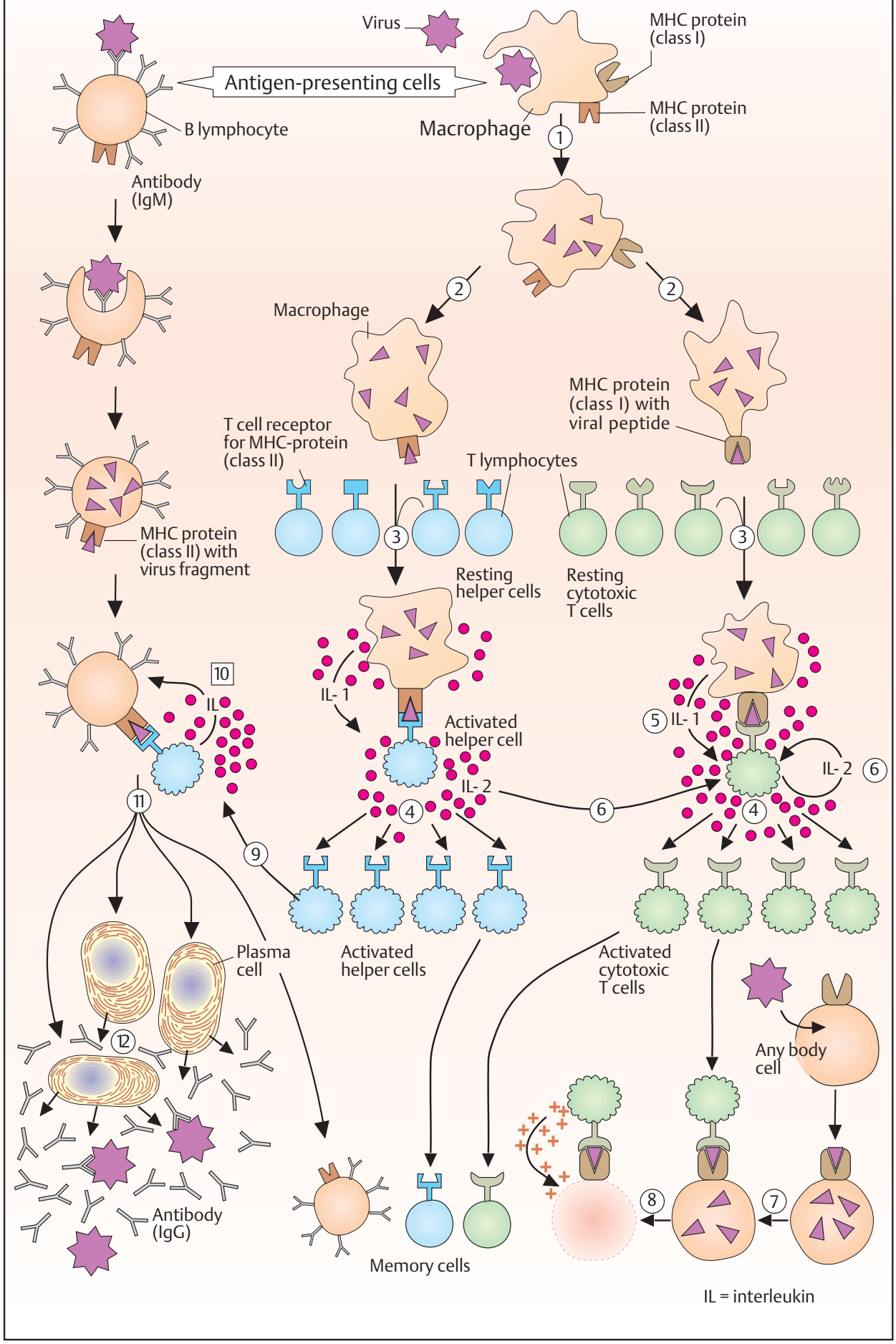
Pathogens that have entered the body—e. g., viruses (top)—are taken up by **antigen-presenting cells** (APCs) and proteolytically degraded (**1**). The viral fragments produced in this way are then presented on the surfaces of these cells with the help of special membrane proteins (MHC proteins; see p. 296) (**2**). The APCs include B lymphocytes, macrophages, and dendritic cells such as the skin's Langerhans cells.

The complexes of MHC proteins and viral fragments displayed on the APCs are recognized by T cells that carry a receptor that matches the antigen ("T-cell receptors"; see p. 296) (**3**). Binding leads to activation of the T cell concerned and selective replication of it (**4**, *"clonal selection"*). The proliferation of immune cells is stimulated by *interleukins* (IL). These are a group of more than 20 signaling substances belonging to the cytokine family (see p. 392), with the help of which immune cells communicate with each other. For example, activated macrophages release IL-1 (**5**), while T cells stimulate their own replication and that of other immune cells by releasing IL-2 (**6**).

Depending on their type, activated T cells have different functions. **Cytotoxic T cells** (green) are able to recognize and bind virus-infected body cells or tumor cells (**7**). They then drive the infected cells into apoptosis (see p. 396) or kill them with *perforin*, a protein that perforates the target cell's plasma membrane (**8**).

B lymphocytes, which as APCs present viral fragments on their surfaces, are recognized by **helper T cells** (blue) or their T cell receptors (**9**). Stimulated by interleukins, selective clonal replication then takes place of B cells that carry antigen receptors matching those of the pathogen (**10**). These mature into **plasma cells** (**11**) and finally secrete large amounts of soluble **antibodies** (**12**).

A. Simplified scheme of the immune response



T-cell activation

For the selectivity of the immune response (see p. 294), the cells involved must be able to recognize foreign antigens and proteins on other immune cells safely and reliably. To do this, they have antigen receptors on their cell surfaces and co-receptors that support recognition.

A. Antigen receptors ○

Many antigen receptors belong to the **immunoglobulin superfamily**. The common characteristic of these proteins is that they are made up from "immunoglobulin domains." These are characteristically folded substructures consisting of 70–110 amino acids, which are also found in soluble immunoglobulins (Ig; see p. 300). The illustration shows schematically a few of the important proteins in the Ig superfamily. They consist of constant regions (brown or green) and variable regions (orange). Homologous domains are shown in the same colors in each case. All of the receptors have transmembrane helices at the C terminus, which anchor them to the membranes. Intramolecular and intermolecular disulfide bonds are also usually found in proteins belonging to the Ig family.

Immunoglobulin M (IgM), a membrane protein on the surface of B lymphocytes, serves to bind free antigens to the B cells. By contrast, **T cell receptors** only bind antigens when they are presented by another cell as a complex with an MHC protein (see below). Interaction between MHC-bound antigens and T cell receptors is supported by **co-receptors**. This group includes **CD8**, a membrane protein that is typical in cytotoxic T cells. T helper cells use **CD4** as a co-receptor instead (not shown). The abbreviation "CD" stands for "cluster of differentiation." It is the term for a large group of proteins that are all located on the cell surface and can therefore be identified by antibodies. In addition to CD4 and CD8, there are many other co-receptors on immune cells (not shown).

The **MHC proteins** are named after the *"major histocompatibility complex"*—the DNA segment that codes for them. Human MHC proteins are also known as HLA antigens ("human leukocyte-associated" antigens). Their polymorphism is so large that it is unlikely that any two individuals carry the same set of MHC proteins—except for monozygotic twins.

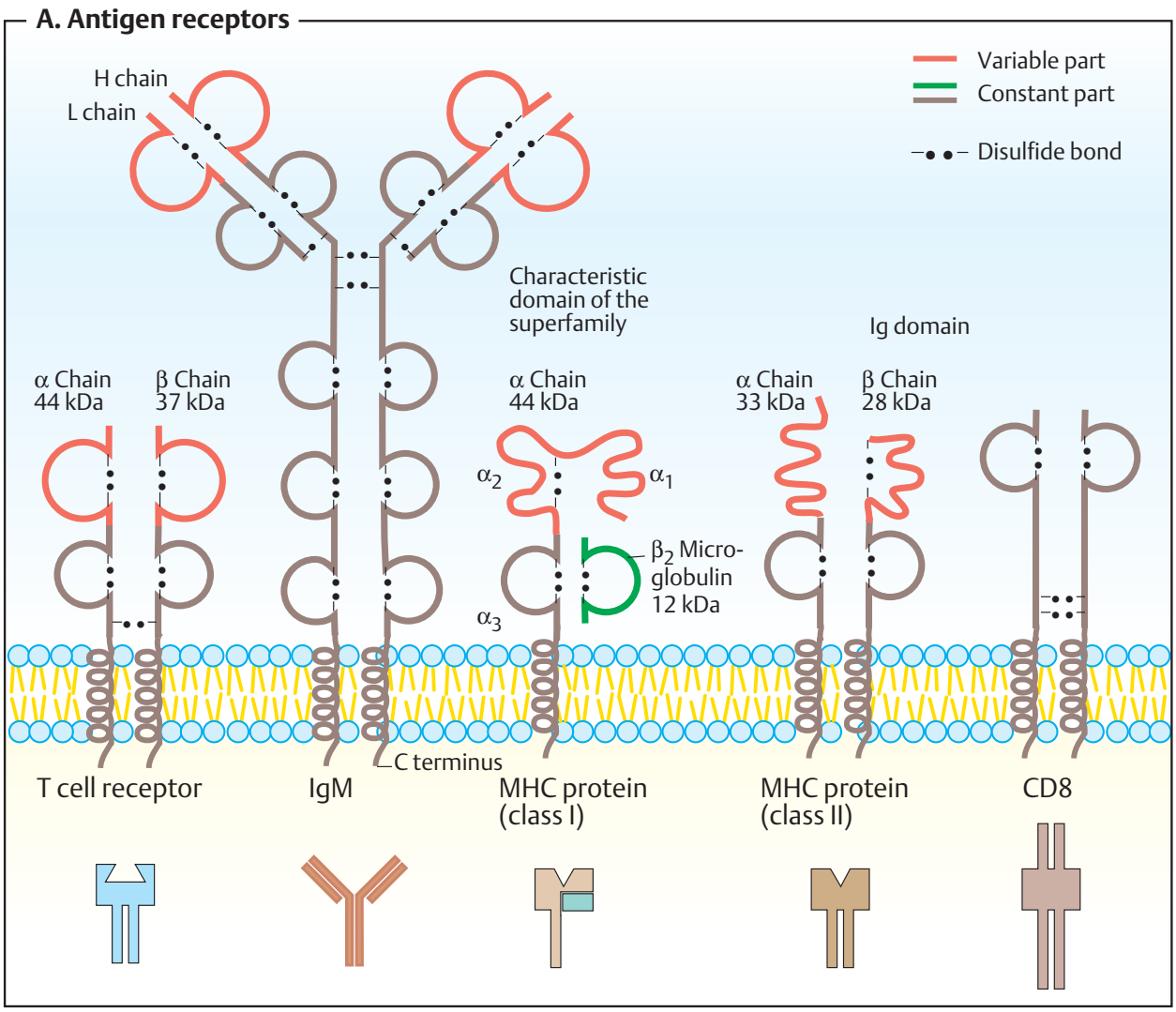
Class I MHC proteins occur in almost all nucleated cells. They mainly interact with cytotoxic T cells and are the reason for the rejection of transplanted organs. Class I MHC proteins are heterodimers ($\alpha\beta$). The β subunit is also known as β_2-microglobulin.

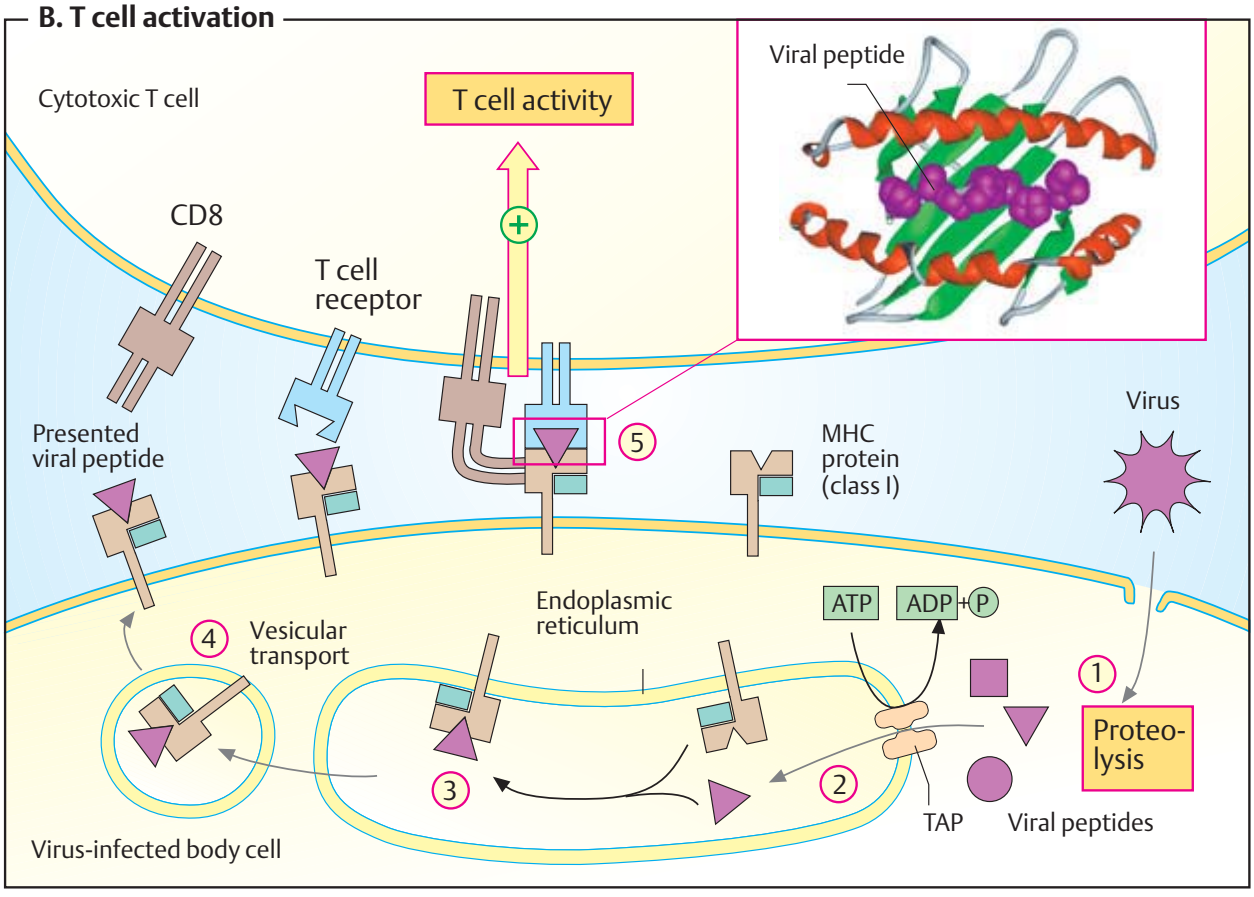
Class II MHC proteins also consist of two peptide chains, which are related to each other. MHC II molecules are found on all antigen-presenting cells in the immune system. They serve for interaction between these cells and CD4-carrying T helper cells.

B. T-cell activation ◑

The illustration shows an interaction between a virus-infected body cell (bottom) and a CD8-carrying cytotoxic T lymphocyte (top). The infected cell breaks down viral proteins in its cytoplasm (**1**) and transports the peptide fragments into the endoplasmic reticulum with the help of a special transporter (*TAP*) (**2**). Newly synthesized class I MHC proteins on the endoplasmic reticulum are loaded with one of the peptides (**3**) and then transferred to the cell surface by vesicular transport (**4**). The viral peptides are bound on the surface of the α_2 domain of the MHC protein in a depression formed by an insertion as a "floor" and two helices as "walls" (see smaller illustration).

Supported by CD8 and other co-receptors, a T cell with a matching T cell receptor binds to the MHC peptide complex (**5**; cf. p. 224). This binding activates protein kinases in the interior of the T cell, which trigger a chain of additional reactions (*signal transduction;* see p. 388). Finally, destruction of the virus-infected cell by the cytotoxic T lymphocytes takes place.





Complement system

The complement system is part of the innate immune system (see p. 294). It supports *nonspecific defense* against microorganisms. The system consists of some 30 different proteins, the *"complement factors,"* which are found in the blood and represent about 4% of all plasma proteins there. When inflammatory reactions occur, the complement factors enter the infected tissue and take effect there.

The complement system works in three different ways:

Chemotaxis. Various complement factors attract immune cells that can attack and phagocytose pathogens.

Opsonization. Certain complement factors ("opsonins") bind to the pathogens and thereby mark them as targets for phagocytosing cells (e. g., macrophages).

Membrane attack. Other complement factors are deposited in the bacterial membrane, where they create pores that lyse the pathogen (see below).

A. Complement activation ◑

The reactions that take place in the complement system can be initiated in several ways. During the early phase of infection, lipopolysaccharides and other structures on the surface of the pathogens trigger the *alternative pathway* (right). If antibodies against the pathogens become available later, the antigen–antibody complexes formed activate the *classic pathway* (left). Acute-phase proteins (see p. 276) are also able to start the complement cascade (*lectin pathway,* not shown).

Factors C1 to **C4** (for "complement") belong to the classic pathway, while **factors B** and **D** form the reactive components of the alternative pathway. Factors **C5** to **C9** are responsible for membrane attack. Other components not shown here regulate the system.

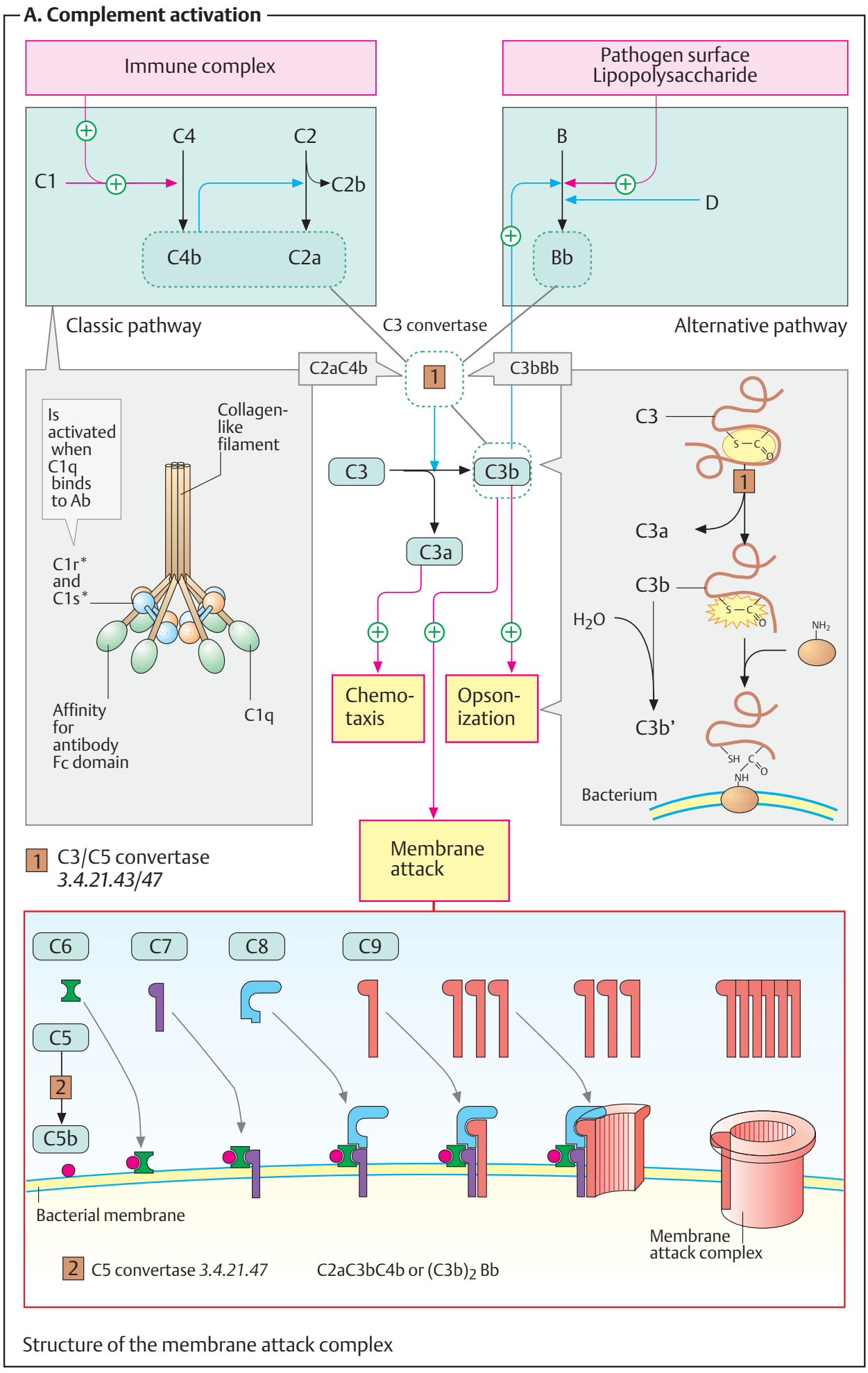
As in blood coagulation (see p. 290), the *early components* in the complement system are *serine proteinases,* which mutually activate each other through limited proteolysis. They create a self-reinforcing **enzyme cascade**. Factor **C3**, the products of which are involved in several functions, is central to the complement system.

The **classic pathway** is triggered by the formation of factor C1 at IgG or IgM on the surface of microorganisms (left). C1 is an 18-part molecular complex with three different components (C1q, C1r, and C1s). C1q is shaped like a bunch of tulips, the "flowers" of which bind to the F_c region of antibodies (left). This activates C1r, a *serine proteinase* that initiates the cascade of the classic pathway. First, C4 is proteolytically activated into C4b, which in turn cleaves C2 into C2a and C2b. C4B and C2a together form *C3 convertase* [1], which finally catalyzes the cleavage of C3 into C3a and C3b. Small amounts of C3b also arise from non-enzymatic hydrolysis of C3.

The **alternative pathway** starts with the binding of factors C3b and B to bacterial lipopolysaccharides (endotoxins). The formation of this complex allows cleavage of B by factor D, giving rise to a second form of *C3 convertase (C3bBb).*

Proteolytic cleavage of factor **C3** provides two components with different effects. The reaction exposes a highly *reactive thioester group* in C3b, which reacts with hydroxyl or amino groups. This allows C3b to bind covalently to molecules on the bacterial surface (*opsonization,* right). In addition, C3b initiates a chain of reactions leading to the formation of the *membrane attack complex* (see below). Together with C4a and C5a (see below), the smaller product C3a promotes the inflammatory reaction and has chemotactic effects.

The "late" factors C5 to C9 are responsible for the development of the **membrane attack complex** (bottom). They create an ion-permeable pore in the bacterial membrane, which leads to lysis of the pathogen. This reaction is triggered by *C5 convertase* [2]. Depending on the type of complement activation, this enzyme has the structure *C4b2a3b* or *C3bBb3b,* and it cleaves C5 into C5a and C5b. The complex of C5b and C6 allows deposition of C7 in the bacterial membrane. C8 and numerous C9 molecules—which form the actual pore—then bind to this core.



Structure of the membrane attack complex

Antibodies

Soluble antigen receptors, which are formed by activated B cells (plasma cells; see p. 294) and released into the blood, are known as **antibodies**. They are also members of the immunoglobulin family (Ig; see p. 296). Antibodies are an important part of the humoral immune defense system. They have no antimicrobial properties themselves, but support the cellular immune system in various ways:

1. They bind to antigens on the surface of pathogens and thereby prevent them from interacting with body cells (*neutralization;* see p. 404, for example).

2. They link single-celled pathogens into aggregates (immune complexes), which are more easily taken up by phagocytes (*agglutination*).

3. They activate the complement system (see p. 298) and thereby promote the innate immune defense system (*opsonization*).

In addition, antibodies have become indispensable aids in medical and biological diagnosis (see p. 304).

A. Domain structure of immunoglobulin G ◑

Type G immunoglobulins (**IgG**) are quantitatively the most important antibodies in the blood, where they form the fraction of γ-globulins (see p. 276). IgGs (mass 150 kDa) are tetramers with two **heavy chains** (H chains; red or orange) and two **light chains** (L chains; yellow). Both H chains are glycosylated (violet; see also p. 43).

The proteinase *papain* cleaves IgG into two F_{ab} fragments and one F_c fragment. The **F_{ab}** ("antigen-binding") fragments, which each consist of one L chain and the N-terminal part of an H chain, are able to bind antigens. The **F_c** ("crystallizable") fragment is made up of the C-terminal halves of the two H chains. This segment serves to bind IgG to cell surfaces, for interaction with the complement system and antibody transport.

Immunoglobulins are constructed in a modular fashion from several **immunoglobulin domains** (shown in the diagram on the right in Ω form). The H chains of IgG contain four of these domains (**V_H**, **C_H1**, **C_H2**, and **C_H3**) and the L chains contain two (**C_L** and **V_L**). The letters C and V designate constant or variable regions.

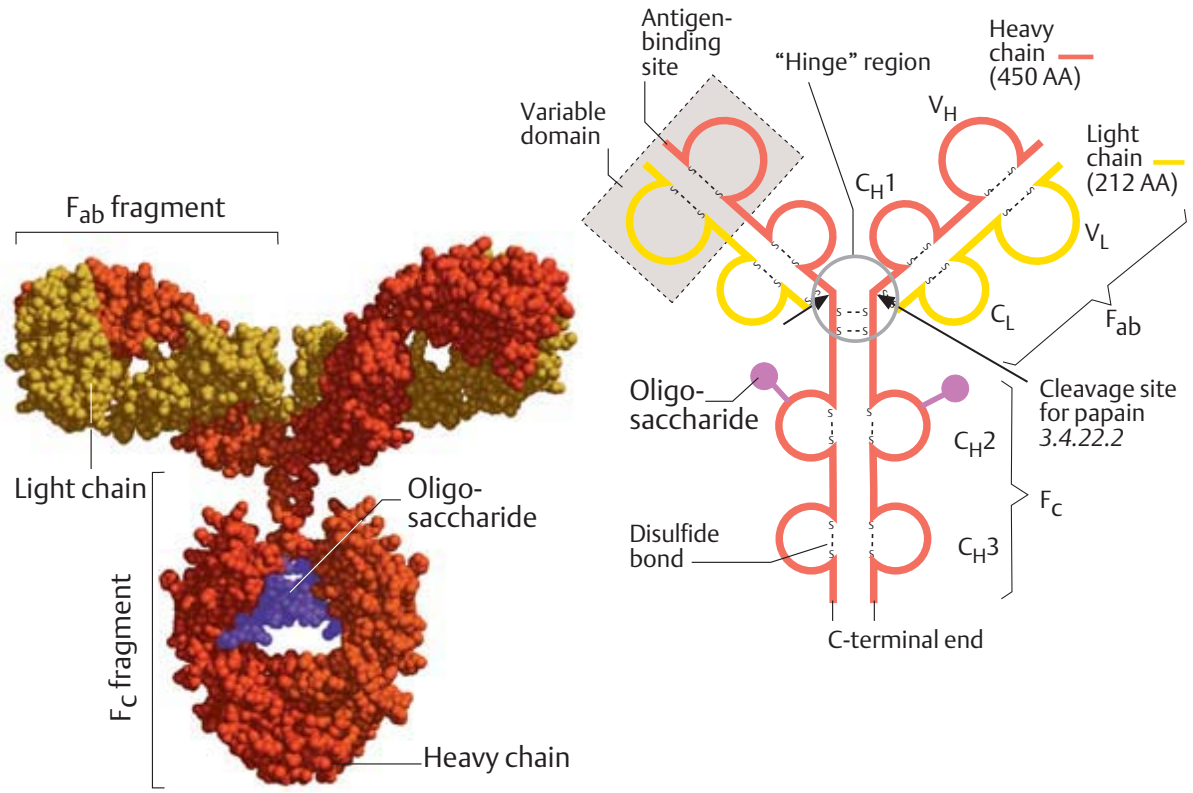
Disulfide bonds link the two heavy chains to each other and also link the heavy chains to the light chains. Inside the domains, there are also disulfide bonds that stabilize the tertiary structure. The domains are approximately 110 amino acids (AA) long and are homologous with each other. The antibody structure evidently developed as a result of gene duplication. In its central region, known as the "hinge" region, the antibodies are highly mobile.

B. Classes of immunoglobulins ◑

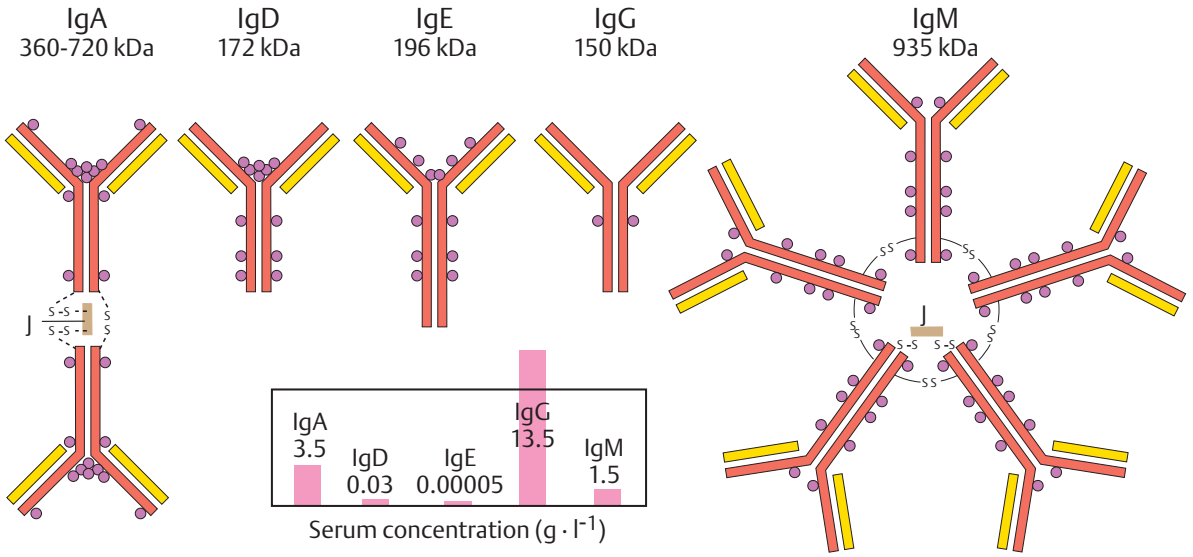
Human immunoglobulins are divided into five classes. **IgA** (with two subgroups), **IgD, IgE, IgG** (with four subgroups), and **IgM** are defined by their H chains, which are designated by the Greek letters α, δ, ε, γ, and μ. By contrast, there are only two types of **L chain** (κ and λ). IgD and IgE (like IgG) are tetramers with the structure H_2L_2. By contrast, soluble IgA and IgM are multimers that are held together by disulfide bonds and additional **J peptides** (joining peptides).

The antibodies have different tasks. **IgMs** are the first immunoglobulins formed after contact with a foreign antigen. Their early forms are located on the surface of B cells (see p. 296), while the later forms are secreted from plasma cells as pentamers. Their action targets microorganisms in particular. Quantitatively, **IgGs** are the most important immunoglobulins (see the table showing serum concentrations). They occur in the blood and interstitial fluid. As they can pass the placenta with the help of receptors, they can be transferred from mother to fetus. **IgAs** mainly occur in the intestinal tract and in body secretions. **IgEs** are found in low concentrations in the blood. As they can trigger degranulation of mast cells (see p. 380), they play an important role in allergic reactions. The function of **IgDs** is still unexplained. Their plasma concentration is also very low.

A. Domain structure of immunoglobulin G



B. Classes of immunoglobulins



Chain:					
H	α	δ	ε	γ	μ
L	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
Structure:					
	$(\alpha_2 \kappa_2)_n J$	$\delta_2 \kappa_2$	$\varepsilon_2 \kappa_2$	$\gamma_2 \kappa_2$	$(\mu_2 \kappa_2)_5 J$
	$(\alpha_2 \lambda_2)_n J$	$\delta_2 \lambda_2$	$\varepsilon_2 \lambda_2$	$\gamma_2 \lambda_2$	$(\mu_2 \lambda_2)_5 J$
	n = 1, 2 or 3				

Antibody biosynthesis

The acquired (adaptive) immune system (see p. 294) is based on the ability of the lymphocytes to keep an extremely large repertoire of antigen receptors and soluble antibodies ready for use, so that even infections involving new types of pathogen can be combated. The wide range of immunoglobulins (Ig) are produced by genetic recombination and additional mutations during the development and maturation of the individual lymphocytes.

A. Variability of immunoglobulins ◑

It is estimated that more than 10^8 different antibody variants occur in every human being. This **variability** affects both the heavy and the light chains of immunoglobulins.

There are five different types of heavy (H) chain, according to which the antibody classes are defined (α, δ, ε, γ, μ), and two types of light (L) chain (κ and λ; see p. 300). The various Ig types that arise from combinations of these chains are known as **isotypes**. During immunoglobulin biosynthesis, plasma cells can switch from one isotype to another ("gene switch"). **Allotypic variation** is based on the existence of various alleles of the same gene—i. e., genetic differences between individuals. The term **idiotypic variation** refers to the fact that the antigen binding sites in the F_{ab} fragments can be highly variable. Idiotypic variation affects the *variable domains* (shown here in pink) of the light and heavy chains. At certain sites—known as the *hypervariable regions* (shown here in red)—variation is particularly wide; these sequences are directly involved in the binding of the antigen.

B. Causes of antibody variety ◑

There are three reasons for the extremely wide variability of antibodies:

1. **Multiple genes.** Various genes are available to code for the variable protein domains. Only one gene from among these is selected and expressed.

2. **Somatic recombination.** The genes are divided into several segments, of which there are various versions. Various ("untidy") combinations of the segments during lymphocyte maturation give rise to randomly combined new genes ("mosaic genes").

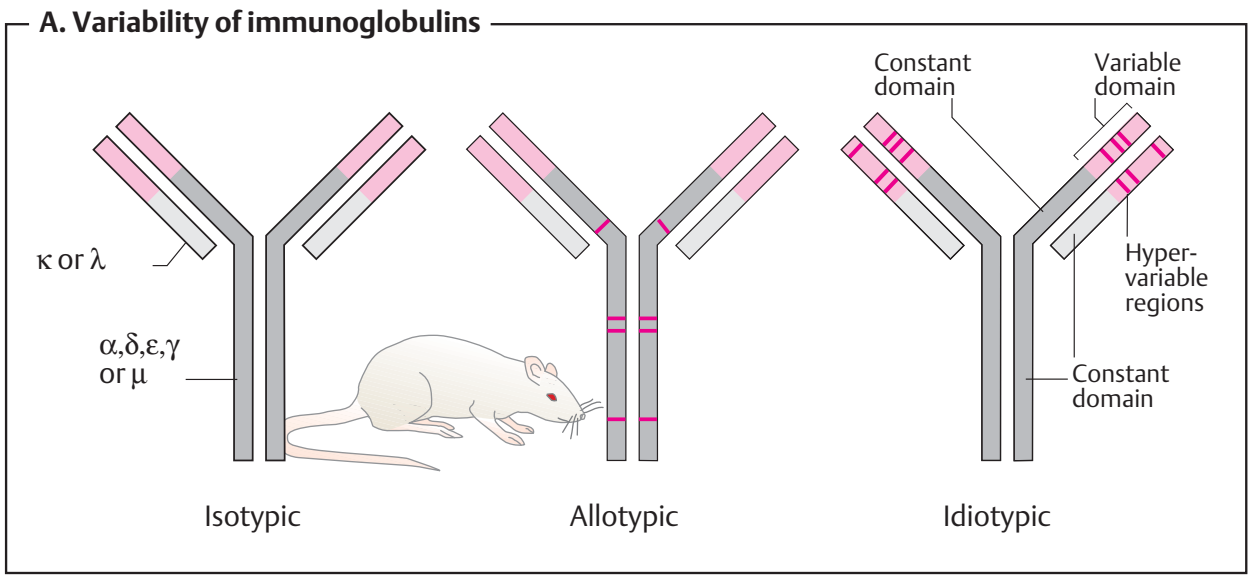
3. **Somatic mutation.** During differentiation of B cells into plasma cells, the coding genes mutate. In this way, the "primordial" *germline genes* can become different *somatic genes* in the individual B cell clones.

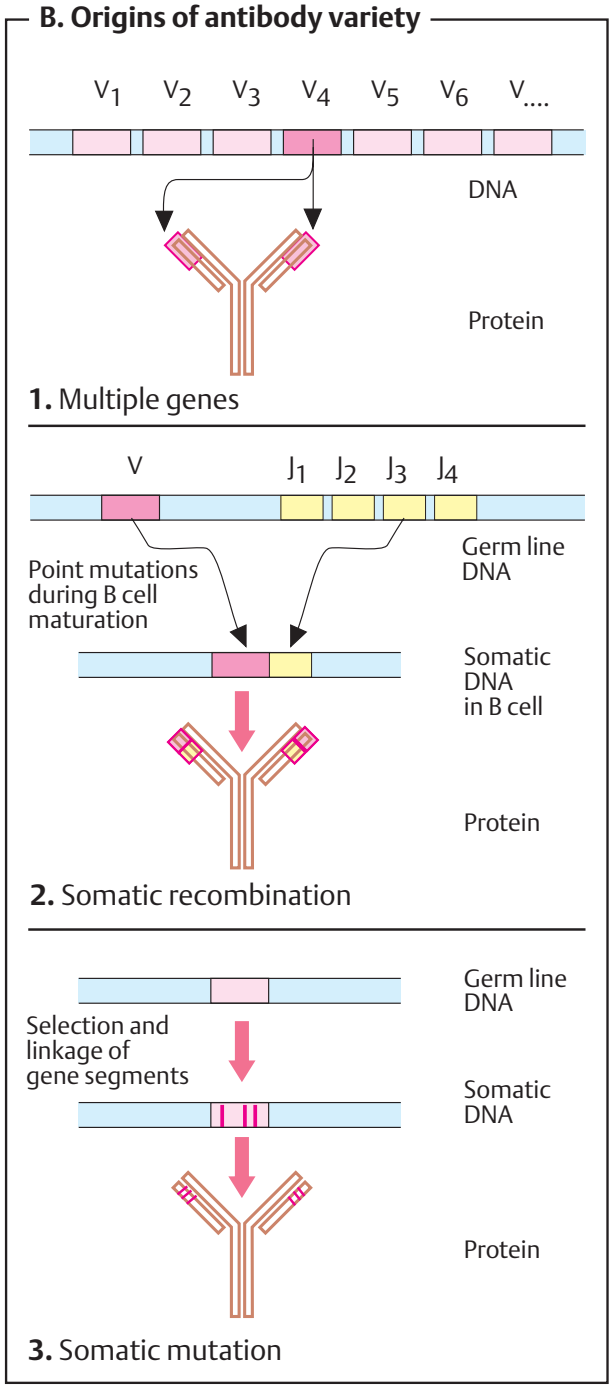
C. Biosynthesis of a light chain ○

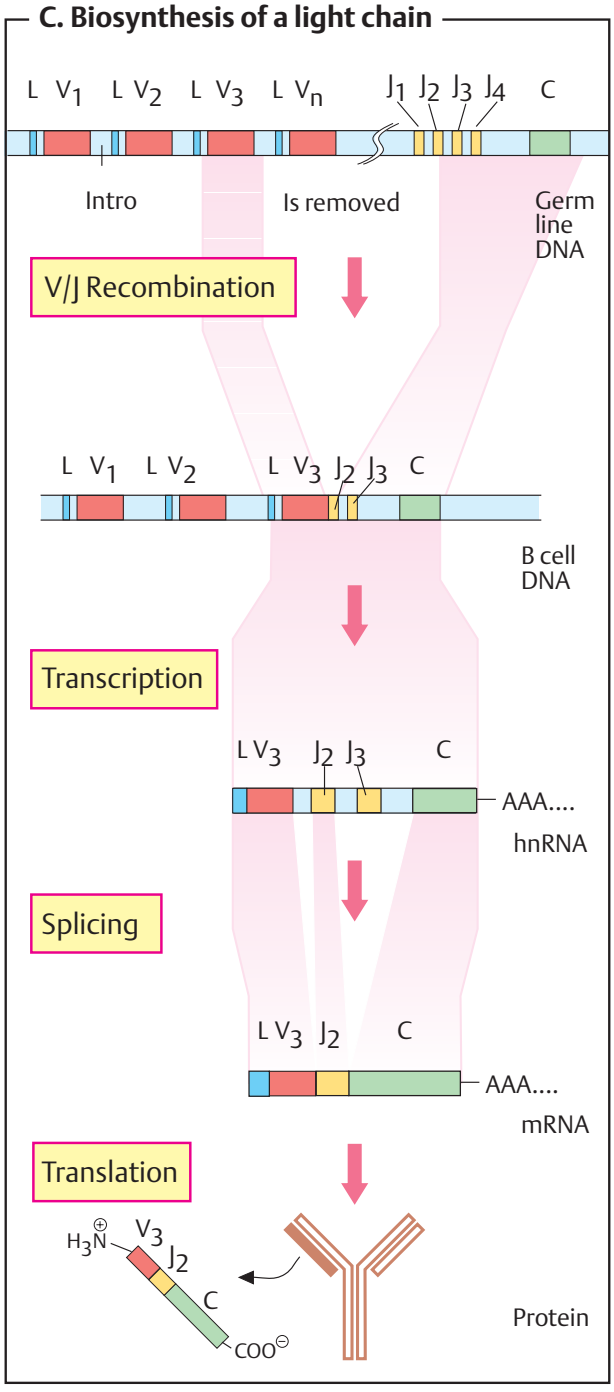
We can look at the basic features of the genetic organization and synthesis of immunoglobulins using the biosynthesis of a mouse κ chain as an example. The gene segments for this light chain are designated L, V, J, and C. They are located on chromosome 6 in the **germ-line DNA** (on chromosome 2 in humans) and are separated from one another by introns (see p. 242) of different lengths.

Some 150 identical **L segments** code for the signal peptide ("leader sequence," 17–20 amino acids) for secretion of the product (see p. 230). The **V segments**, of which there are 150 different variants, code for most of the variable domains (95 of the 108 amino acids). L and V segments always occur in pairs—in tandem, so to speak. By contrast, there are only five variants of the **J segments** (joining segments) at most. These code for a peptide with 13 amino acids that links the variable part of the κ chains to the constant part. A single **C segment** codes for the constant part of the light chain (84 amino acids).

During the differentiation of B lymphocytes, individual *V/J combinations* arise in each B cell. One of the 150 L/V tandem segments is selected and linked to one of the five J segments. This gives rise to a *somatic gene* that is much smaller than the germline gene. Transcription of this gene leads to the formation of the **hnRNA** for the κ chain, from which introns and surplus J segments are removed by splicing (see p. 246). Finally, the completed **mRNA** still contains one each of the L–V–J–C segments and after being transported into the cytoplasm is available for translation. The subsequent steps in Ig biosynthesis follow the rules for the synthesis of membrane-bound or secretory proteins (see p. 230).







Monoclonal antibodies, immunoassay

A. Monoclonal antibodies ○

Monoclonal antibodies (MABs) are secreted by immune cells that derive from a single antibody-forming cell (from a single cell *clone*). This is why each MAB is directed against only one specific *epitope* of an immunogenic substance, known as an *"antigenic determinant."* Large molecules contain several epitopes, against which various antibodies are formed by various B cells. An antiserum containing a mixture of all of these antibodies is described as being *polyclonal.*

To obtain MABs, **lymphocytes** isolated from the spleen of immunized mice (**1**) are fused with mouse tumor cells (**myeloma cells**, **2**). This is necessary because antibody-secreting lymphocytes in culture have a lifespan of only a few weeks. Fusion of lymphocytes with tumor cells gives rise to cell hybrids, known as **hybridomas,** which are potentially immortal.

Successful *fusion* (**2**) is a rare event, but the frequency can be improved by adding polyethylene glycol (PEG). To obtain only successfully fused cells, incubation is required for an extended period in a **primary culture** with *HAT medium* (**3**), which contains hypoxanthine, aminopterin, and thymidine. *Aminopterin*, an analogue of dihydrofolic acid, competitively inhibits *dihydrofolate reductase* and thus inhibits the synthesis of dTMP (see p. 402). As dTMP is essential for DNA synthesis, myeloma cells cannot survive in the presence of aminopterin. Although spleen cells are able to circumvent the inhibitory effect of aminopterin by using *hypoxanthine* and *thymidine,* they have a limited lifespan and die. Only hybridomas survive culture in HAT medium, because they possess both the immortality of the myeloma cells and the spleen cells' metabolic side pathway.

Only a few fused cells actually produce antibodies. To identify these cells, the hybridomas have to be isolated and replicated by **cloning** (**4**). After the clones have been tested for antibody formation, positive cultures are picked out and selected by further cloning (**5**). This results in hybridomas that synthesize *monoclonal antibodies.* Finally, MAB production is carried out in vitro using a bioreactor, or in vivo by producing ascites fluid in mice (**6**).

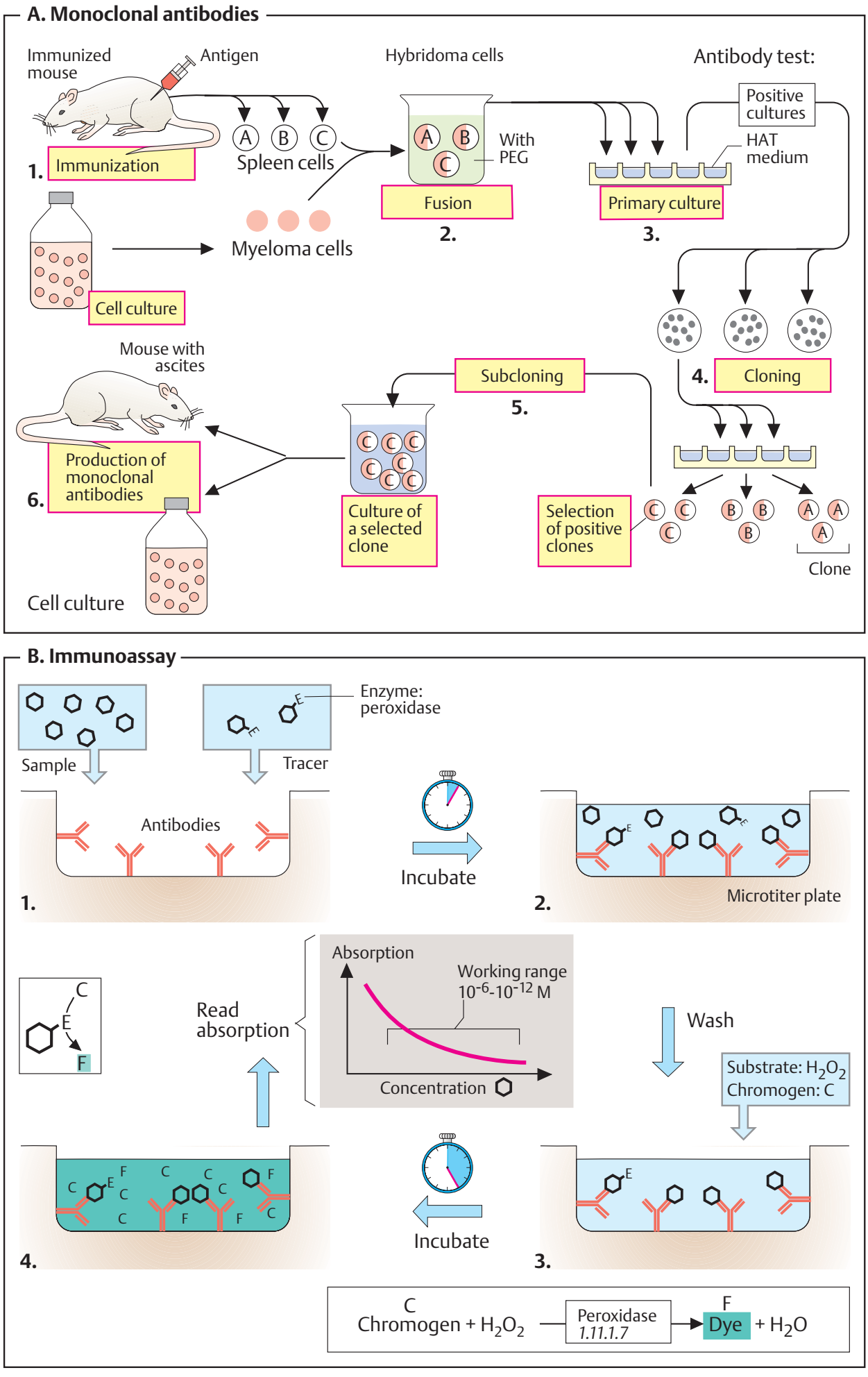
B. Immunoassay ○

Immunoassays are semiquantitative procedures for assessing substances with low concentrations. In principle, immunoassays can be used to assess any compound against which antibodies are formed.

The basis for this procedure is the *antigen–antibody "reaction"*—i. e., specific binding of an antibody to the molecule being assayed. Among the many different immunoassay techniques that have been developed—e. g., *radioimmunoassay* (RIA), and *chemoluminescence immunoassay* (CIA)—a version of the **enzyme-linked immunoassay** (EIA) is shown here.

The substance to be assayed—e. g., the hormone thyroxine in a serum sample—is pipetted into a microtiter plate (**1**), the walls of which are coated with *antibodies* that specifically bind the hormone. At the same time, a small amount of thyroxine is added to the incubation to which an enzyme known as the *"tracer"* (**1**) has been chemically coupled. The tracer and the hormone being assayed compete for the small number of antibody binding sites available. After binding has taken place (**2**), all of the unbound molecules are rinsed out. The addition of a substrate solution for the enzyme (a *chromogenic solution*) then triggers an indicator reaction (**3**), the products of which can be assessed using photometry (**4**).

The larger the amount of enzyme that can bind to the antibodies on the container's walls, the larger the amount of dye that is produced. Conversely, the larger the amount of the substance being assayed that is present in the sample, the *smaller* the amount of tracer that can be bound by the antibodies. Quantitative analysis can be carried out through parallel measurement using standards with a known concentration.



Liver: functions

Weighing 1.5 kg, the liver is one of the largest organs in the human body. Although it only represents 2–3% of the body's mass, it accounts for 25–30% of oxygen consumption.

A. Diagram of a hepatocyte ◑

The 3 10^{11} cells in the liver—particularly the **hepatocytes**, which make up 90% of the cell mass—are the central location for the body's *intermediary metabolism.* They are in close contact with the blood, which enters the liver from the portal vein and the hepatic arteries, flows through capillary vessels known as sinusoids, and is collected again in the central veins of the hepatic lobes. Hepatocytes are particularly rich in endoplasmic reticulum, as they carry out intensive protein and lipid synthesis. The cytoplasm contains granules of insoluble glycogen. Between the hepatocytes, there are bile capillaries through which bile components are excreted.

B. Functions of the liver ●

The most important functions of the liver are:

1. **Uptake** of nutrients supplied by the intestines via the portal vein.
2. Biosynthesis of endogenous compounds and storage, conversion, and degradation of them into excretable molecules (**metabolism**). In particular, the liver is responsible for the biosynthesis and degradation of almost all plasma proteins.
3. **Supply** of the body with metabolites and nutrients.
4. **Detoxification** of toxic compounds by biotransformation.
5. **Excretion** of substances with the bile.

C. Hepatic metabolism ●

The liver is involved in the metabolism of practically all groups of metabolites. Its functions primarily serve to cushion fluctuations in the concentration of these substances in the blood, in order to ensure a constant supply to the peripheral tissues (*homeostasis*).

Carbohydrate metabolism. The liver takes up glucose and other monosaccharides from the plasma. Glucose is then either stored in the form of the polysaccharide *glycogen* or converted into fatty acids. When there is a drop in the blood glucose level, the liver releases glucose again by breaking down glycogen. If the glycogen store is exhausted, glucose can also be synthesized by *gluconeogenesis* from lactate, glycerol, or the carbon skeleton of amino acids (see p. 310).

Lipid metabolism. The liver synthesizes fatty acids from acetate units. The fatty acids formed are then used to synthesize fats and phospholipids, which are released into the blood in the form of *lipoproteins.* The liver's special ability to convert fatty acids into *ketone bodies* and to release these again is also important (see p. 312).

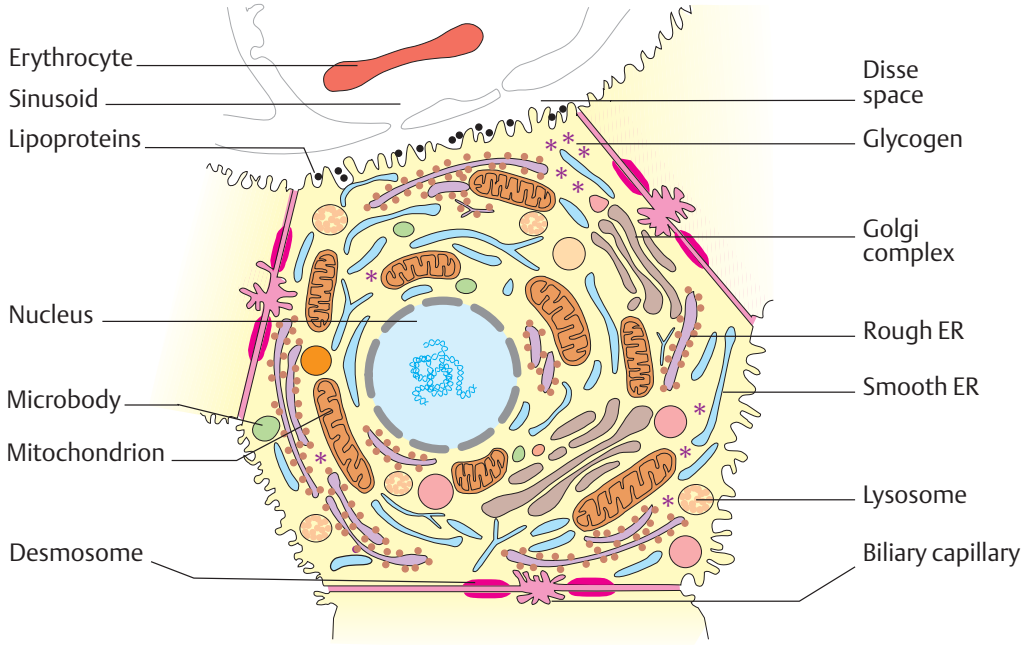
Like other organs, the liver also synthesizes cholesterol, which is transported to other tissues as a component of lipoproteins. Excess cholesterol is converted into bile acids in the liver or directly excreted with the bile (see p. 314).

Amino acid and protein metabolism. The liver controls the plasma levels of the amino acids. Excess amino acids are broken down. With the help of the urea cycle (see p. 182), the nitrogen from the amino acids is converted into urea and excreted via the kidneys. The carbon skeleton of the amino acids enters the intermediary metabolism and serves for glucose synthesis or energy production. In addition, most of the plasma proteins are synthesized or broken down in the liver (see p. 276).

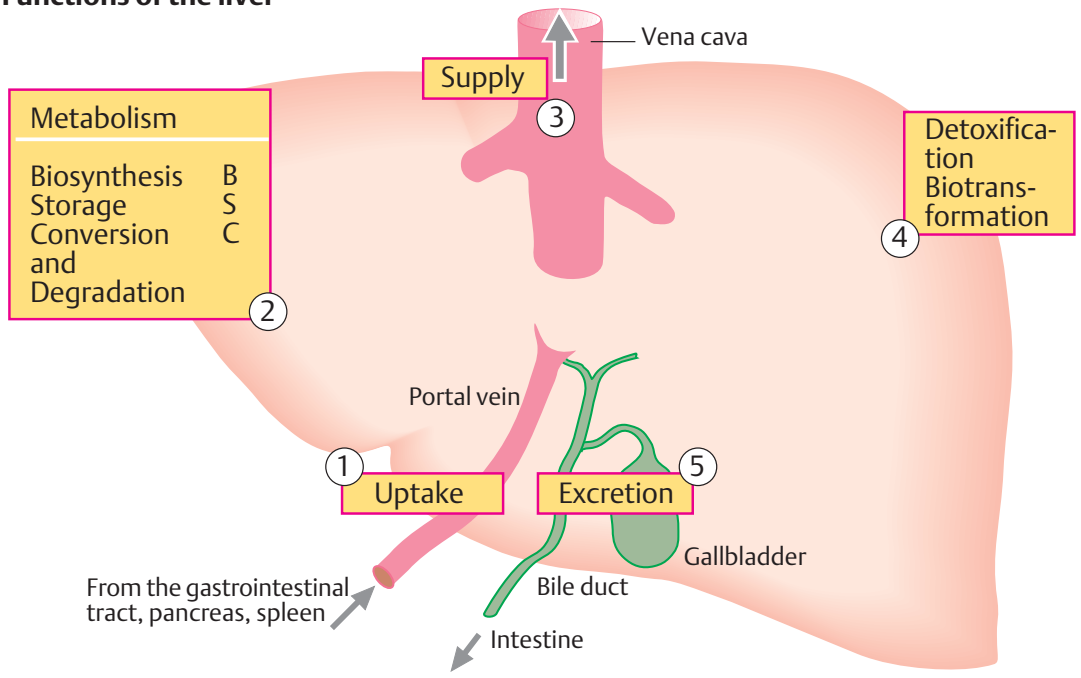
Biotransformation. Steroid hormones and bilirubin, as well as drugs, ethanol, and other xenobiotics are taken up by the liver and inactivated and converted into highly polar metabolites by conversion reactions (see p. 316).

Storage. The liver not only stores energy reserves and nutrients for the body, but also certain mineral substances, trace elements, and vitamins, including iron, retinol, and vitamins A, D, K, folic acid, and B_{12}.

A. Diagram of a hepatocyte



B. Functions of the liver



C. Liver metabolism

Carbohydrate metabolism	
Glucose	BSC
Galactose	C
Fructose	C
Mannose	C
Pentoses	BC
Lactate	C
Glycerol	BC
Glycogen	BSC

Lipid metabolism	
Fatty acids	BC
Fats	BC
Ketone bodies	B
Cholesterol	BEC
Bile acids	BE
Vitamins	SC

Amino acid metabolism	
Amino acids	C
Urea	B

Plasma proteins	
Lipoproteins	BC
Albumin	BC
Coagulation factors	BC
Hormones	C
Enzymes	BC

Biotransformation	
Steroid hormones	EC
Bile pigments	EC
Ethanol	C
Drugs	EC

B	Biosynthesis
C	Conversion and degradation
E	Excretion
S	Storage

Buffer function in organ metabolism

All of the body's tissues have a constant requirement for energy substrates and nutrients. The body receives these metabolites with food, but the supply is irregular and in varying amounts. The liver acts here along with other organs, particularly adipose tissue, as a balancing *buffer* and *storage organ.*

In the metabolism, a distinction is made between the *absorptive state (well-fed state)* immediately after a meal and the *postabsorbtive state (state of starvation)*, which starts later and can merge into hunger. The switching of the organ metabolism between the two phases depends on the concentration of energy-bearing metabolites in the blood (plasma level). This is regulated jointly by hormones and by the autonomic nervous system.

A. Absorptive state ◑

The absorptive state continues for 2–4 hours after food intake. As a result of food digestion, the plasma levels of glucose, amino acids, and fats (triacylglycerols) temporarily increase.

The endocrine **pancreas** responds to this by altering its hormone release—there is an increase in *insulin* secretion and a reduction in *glucagon* secretion. The increase in the insulin/glucagon quotient and the availability of substrates trigger an *anabolic phase* in the tissues—particularly liver, muscle, and adipose tissues.

The **liver** forms increased amounts of glycogen and fats from the substrates supplied. Glycogen is stored, and the fat is released into the blood in very low density lipoproteins (VLDLs).

Muscle also refills its glycogen store and synthesizes proteins from the amino acids supplied.

Adipose tissue removes free fatty acids from the lipoproteins, synthesizes triacylglycerols from them again, and stores these in the form of insoluble droplets.

During the absorptive state, the **heart** and **neural tissue** mainly use glucose as an energy source, but they are unable to establish any substantial energy stores. Heart muscle cells are in a sense "omnivorous," as they can also use other substances to produce energy (fatty acids, ketone bodies). By contrast, the central nervous system (CNS) is dependent on glucose. It is only able to utilize ketone bodies after a prolonged phase of hunger (**B**).

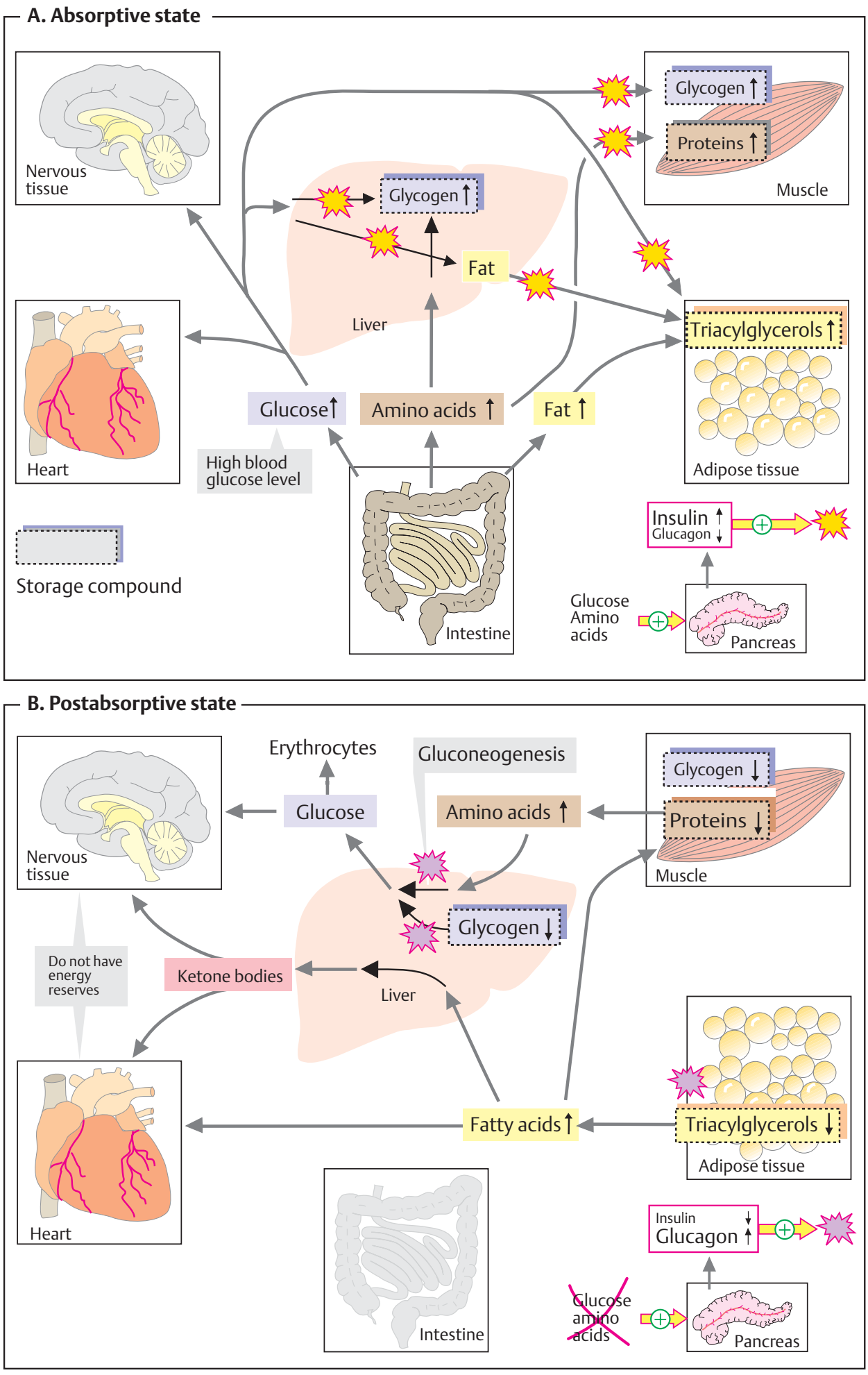
B. Postabsorptive state ◑

When the food supply is interrupted, the postabsorbtive state quickly sets in. The pancreatic A cells now release increased amounts of *glucagon,* while the B cells reduce the amount of *insulin* they secrete. The reduced insulin/glucagon quotient leads to switching of the intermediary metabolism. The body now falls back on its energy reserves. To do this, it breaks down *storage substances* (glycogen, fats, and proteins) and shifts energy-supplying metabolites between the organs.

The **liver** first empties its glycogen store (*glycogenolysis;* see p. 156). It does not use the released glucose itself, however, but supplies the other tissues with it. In particular, the brain, adrenal gland medulla, and erythrocytes depend on a constant supply of glucose, as they have no substantial glucose reserves themselves. When the liver's glycogen reserves are exhausted after 12–24 hours, *gluconeogenesis* begins (see p. 154). The precursors for this are derived from the musculature (amino acids) and adipose tissue (glycerol from fat degradation). From the fatty acids that are released (see below), the liver starts to form ketone bodies (*ketogenesis;* see p. 312). These are released into the blood and serve as important energy suppliers during the hunger phase. After 1–2 weeks, the CNS also starts to use ketone bodies to supply part of its energy requirements, in order to save glucose.

In **muscle**, the extensive glycogen reserves are exclusively used for the muscles' own requirements (see p. 320). The slowly initiated protein breakdown in muscle supplies amino acids for gluconeogenesis in the liver.

In **adipose tissue**, glucagon triggers *lipolysis,* releasing fatty acids and glycerol. The fatty acids are used as energy suppliers by many types of tissue (with the exception of brain and erythrocytes). An important recipient of the fatty acids is the liver, which uses them for ketogenesis.



Carbohydrate metabolism

Besides fatty acids and ketone bodies, glucose is the body's most important energy supplier. The concentration of glucose in the blood (the *"blood glucose level"*) is maintained at 4–6 mM (0.8–1.0 g L^{-1}) by precise regulation of glucosesupplying and glucose-utilizing processes. Glucose suppliers include the intestines (glucose from food), liver, and kidneys. The liver plays the role of a "glucostat" (see p. 308).

The liver is also capable of forming glucose by converting other sugars—e. g., *fructose* and *galactose*—or by synthesizing from other metabolites. The conversion of lactate to glucose in the *Cori cycle* (see p. 338) and the conversion of alanine to glucose with the help of the *alanine cycle* (see p. 338) are particularly important for the supply of erythrocytes and muscle cells.

Transporters in the plasma membrane of hepatocytes allow insulin-independent transport of glucose and other sugars in both directions. In contrast to muscle, the liver possesses the enzyme *glucose-6-phosphatase,* which can release glucose from glucose-6-phosphate.

A. Gluconeogenesis: overview ◑

Regeneration of glucose (up to 250 g per day) mainly takes place in the liver. The tubule cells of the kidney are also capable of carrying out gluconeogenesis, but due to their much smaller mass, their contribution only represents around 10% of total glucose formation. Gluconeogenesis is regulated by hormones. *Cortisol, glucagon,* and *epinephrine* promote gluconeogenesis, while *insulin* inhibits it (see pp. 158, 244).

The main precursors of gluconeogenesis in the liver are *lactate* from anaerobically working muscle cells and from erythrocytes, glucogenic *amino acids* from the digestive tract and muscles (mainly alanine), and *glycerol* from adipose tissue. The kidney mainly uses amino acids for gluconeogenesis (Glu, Gln; see p. 328).

In mammals, fatty acids and other suppliers of acetyl CoA are not capable of being used for gluconeogenesis, as the acetyl residues formed during β-oxidation in the tricarboxylic acid cycle (see p. 132) are oxidized to CO_2 and therefore cannot be converted into oxaloacetic acid, the precursor for gluconeogenesis.

B. Fructose and galactose metabolism ◑

Fructose is mainly metabolized by the liver, which channels it into glycolysis (left half of the illustration).

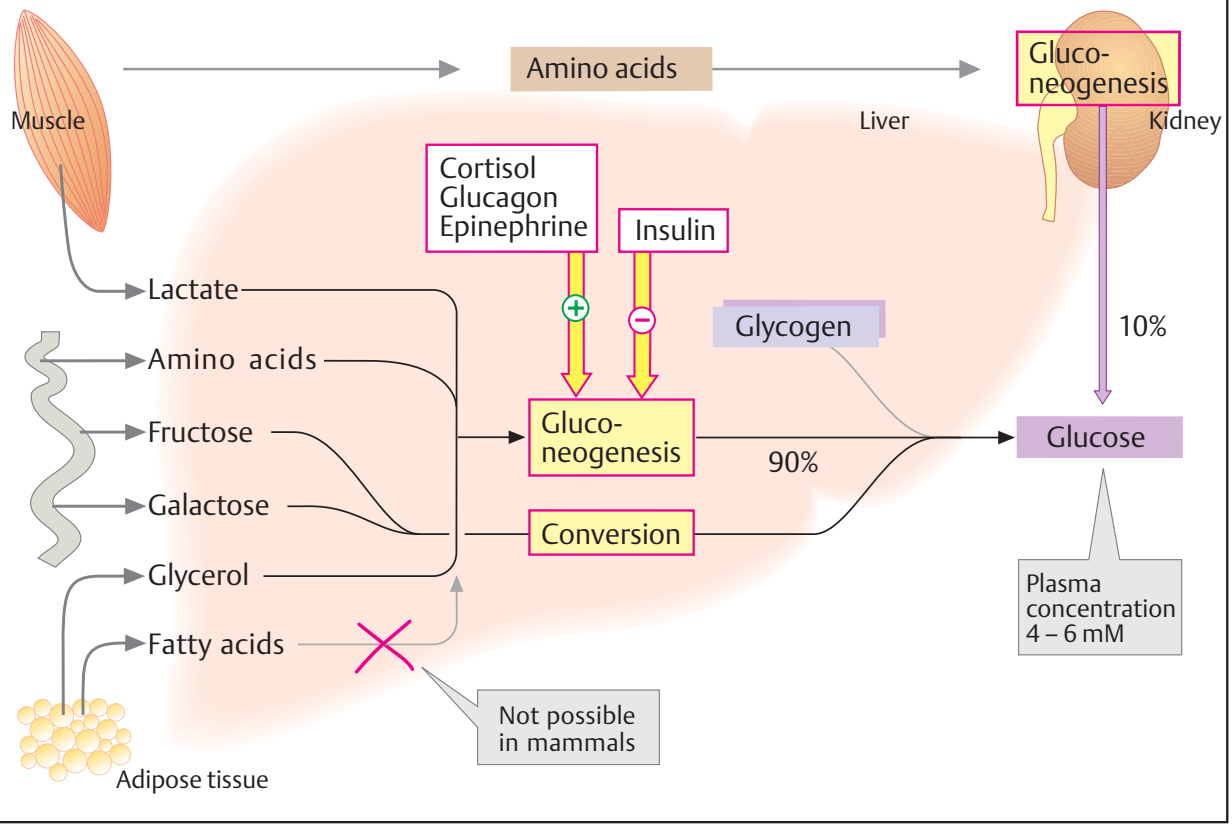
A special *ketohexokinase* [1] initially phosphorylates fructose into **fructose 1-phosphate**. This is then cleaved by an *aldolase* [2], which is also fructose-specific, to yield **glycerone 3-phosphate** (dihydroxyacetone phosphate) and **glyceraldehyde**. Glycerone 3-phosphate is already an intermediate of glycolysis (center), while glyceraldehyde can be phosphorylated into glyceraldehyde 3-phosphate by *triokinase* [3].

To a smaller extent, glyceraldehyde is also reduced to glycerol [4] or oxidized to glycerate, which can be channeled into glycolysis following phosphorylation (not shown). The reduction of glyceraldehyde [4] uses up NADH. As the rate of degradation of alcohol in the hepatocytes is limited by the supply of NAD^+, fructose degradation accelerates alcohol degradation (see p. 320).

Outside of the liver, fructose is channeled into the sugar metabolism by reduction at C-2 to yield sorbitol and subsequent dehydration at C-1 to yield glucose (the *polyol pathway;* not shown).

Galactose is also broken down in the liver (right side of the illustration). As is usual with sugars, the metabolism of galactose starts with a phosphorylation to yield **galactose 1-phosphate** [5]. The connection to the glucose metabolism is established by C-4 epimerization to form **glucose 1-phosphate**. However, this does not take place directly. Instead, a *transferase* [6] transfers a uridine 5′-monophosphate (UMP) residue from uridine diphosphoglucose (UDPglucose) to galactose 1-phosphate. This releases glucose 1-phosphate, while galactose 1-phosphate is converted into uridine diphosphogalactose (UDPgalactose). This then is isomerized into UDPglucose. The *biosynthesis* of galactose also follows this reaction pathway, which is freely reversible up to reaction [5]. Genetic defects of enzymes [5] or [6] can lead to the clinical picture of *galactosemia.*

A. Gluconeogenesis: overview



B. Fructose and galactose metabolism