3
Microbial Metabolism

3.1
Some Remarks on the Composition and Morphology of Bacteria (Eubacteria)

The elementary composition of bacteria does not vary considerably during their life cycle and between different strains. Table 3.1 presents data for *Escherichia coli* (*E. coli*), left side (Bailey and Ollis 1977) and as mean values for bacteria, right side (Schlegel 1992). The various types of bacteria according to the eighth edition of Bergey's Manual of Determinative Bacteriology (1974) are categorized into 19 groups (Cano and Colomé 1986) or in 17 sections and seven groups (Volk and Wheeler 1986). Detailed information about the most important microorganisms was compiled by O'Leary (1989). The importance of microbial communities in environmental microbiology was the subject of the editors Insam and Rangger (1997). Schön (1978a, b) published an introduction for students.

Table 3.1 Composition of bacteria (*E. coli*).

Element	Mass % of dry matter	Substances	Mass % of dry matter
Carbon	50	Inorganics	6
Oxygen	20	Grease	10
Nitrogen	14		
Hydrogen	8	Micromolecular organics (excluding grease)	7
Phosphorus	3		
Potassium	1	Macromolecular organics (polymers)[a)]	77
Sulfur	1		
Sodium	1	Proteins	50
Calcium	0.5	Nucleic acids RNA	16–17
Magnesium	0.5	DNA	3–4
Chlorine	0.5	Lipids	10
Iron	0.2	Cell wall	20
Others	0.3		

[a)] Polymers recalculated to 100%.

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1. Cocci, sperical bacteria

Gram-positive:

• aerobic and/or facultative anaerobic:	Lactobacillus *Streptococcus, Leuconostoc, Pediococcus* *Micrococcus, Staphylococcus*
• anaerobic	*Peptococcus, Peptostreptococcus, Ruminococcu* *Sarcinas*

Gram-negative:

• aerobic	*Neisseria, Moraxella, Acinetobacter,* *Paracoccus, Lampropedia, Methylococcus*
• anaerobic	*Veillonella, Acidaminococcus, Megasphaera*

2. Rod-shaped, stretched cylindrical bacteria

A) Non-spore forming rods

Gram-positive:

• aerobic and/or facultative anaerobic:	Coryneform bacteria *Corynebacterium, Arthrobacter, Brevibacterium,* *Cellulomonas, Mycobacterium, Nocardia*
• anaerobic:	Propionat bacteria Lactobacilla *Lactobacillus, Bifidobacterium*

Gram-negative:

• aerobic:	Pseudomonads *Pseudomonas, Xanthomonas, Zoogloes* *Acetobacter*-Gruppe *Azotobacter*-Gruppe *Azotobacter, Azomonas, Beijerinckia, Derxia*
• facultative anaerobic:	Entero bacteria *Escherichia, Salmonella* *Shigella, Klebsiella, Serratia* *Proteus, Erwinia, Enterobacter* *Chromobacterium, Pasteurella*
• strictly anaerobic:	*Bacteroides, Fusobacterium*

B) Spore forming rods (bacilli)

• aerobic:	*Bacillus, Sporolactobacillus* *Sporosarcina*
• anaerobic:	*Clostridium, Desulfotomaculum* *Oscillospira*

3. Curved rods

• aerobic:	*Spirillum, Vibrio, Bdellovibrio* *Desulfovibrio*
• anaerobic:	*Succinovibrio, Butyrivibrio, Selenomonas*

Fig. 3.1 Eubacteria (Schlegel 1992).

Normally, bacteria are divided into ten classes. The first class is that of the eubacteria, which is the most important in environmental microbiology (Schlegel 1992). Therefore, only eubacteria are characterized here in a general manner by Fig. 3.1.

The size of bacteria in comparison to other particles is demonstrated by Fig. 3.2. Because of their small diameter of only 10^{-3} mm, bacteria have a large surface per mass. Since the uptake of nutrients occurs via the outer surface, bacteria are characterized by a high growth rate, resulting in a high substrate removal rate.

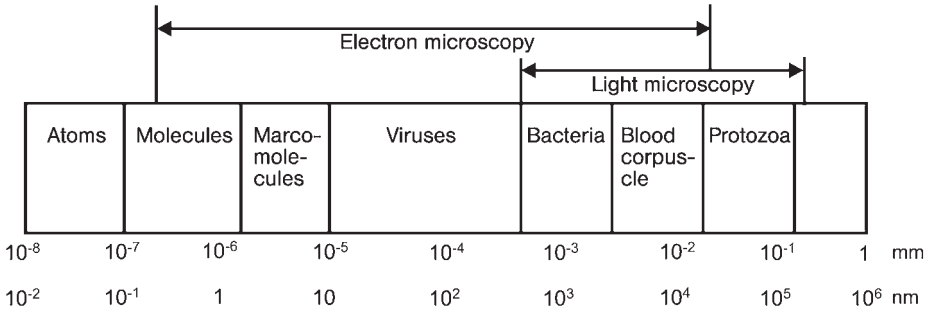

Fig. 3.2 Mean diameters of particles.

3.2
Proteins and Nucleic Acids

3.2.1
Proteins

3.2.1.1 Amino Acids

As follows from Table 3.1, nearly 50% of bacterial cell mass is protein. Proteins are macromolecules formed by a chain of amino acids. Twenty different amino acids are used in nature for building proteins; and they are all characterized by the same groups, called peptides (Fig. 3.3).

The simplest amino acid is formed with only a hydrogen atom located on a side-chain. It is called glycine (Fig. 3.3a). There exist two isomeric types of glycine, an L-type (Fig. 3.3b) which rotates polarized light to the left in aqueous solution and a

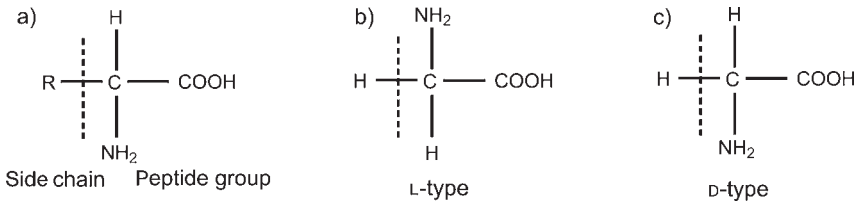

Fig. 3.3 Amino acids. (a) Structure of an amino acid. (b) L-type: isometric type of the simplest amino acid glycine. (c) D-type: isometric type of the simplest amino acid glycine.

Table 3.2 Amino acids used for protein synthesis.

Amino acid	Abbreviation	Side-chain
Cysteine	Cys	Neutral polar (uncharged)
Tyrosine	Tyr	
Asparagine	Asn	
Glutamine	Gln	
Aspartic acid	Asp	Acidic (negatively charged)
Glutamic acid	Glu	
Glycine	Gly	Neutral polar (uncharged)
Serine	Ser	
Threonine	Thr	
Lysine	Lys	Basic (positively charged)
Arginine	Arg	
Histidin	His	
Alanine	Ala	Nonpolar (hydrophobic)
Valine	Val	
Leucine	Leu	
Isoleucine	Ile	
Proline	Pro	
Phenylalanine	Phe	
Tryptophane	Trp	
Methionine	Mat	

D-type, which rotates it to the right (Fig. 3.3c). Only the L-type is used for protein synthesis; the D-type is used for antibody synthesis. The other 19 amino acids have asymmetric side-chains, each with a number of isomeric types. They are shown in Table 3.2.

The chemical structures of all the 20 amino acids are published in several textbooks of microbiology, for example Cano and Colomé (1986) and Gaudy and Gaudy (1989).

3.2.1.2 **Structure of Proteins**

Amino acids polymerize by forming a polypeptide bond and releasing a water molecule, thereby building a protein molecule (Fig. 3.4). The 20 amino acids can be combined to many permutations in this way to form large protein molecules with a molar mass up to 10^6 g mol^{-1} and an enormous number of different successions of amino acids. The four atoms of the peptide bond are oriented in one plane, in contrast to all other atoms which lie in different planes.

The primary structure of the protein molecule results from a linear polymerization. R_1, R_2, ... R_{20} are the different side-chains which are typical for one of the 20 different amino acids.

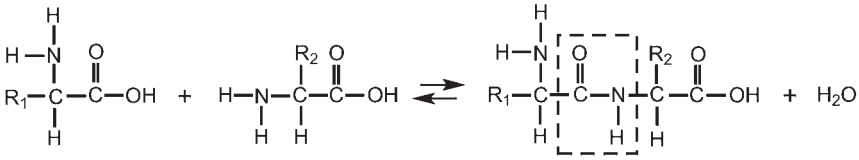

Fig. 3.4 Formation of a peptide bond.

3.2.1.3 Proteins for Special Purposes

Because of the high number of possible structures and varying molar mass, proteins are able to assume different functions. Some of these are described in Table 3.3.

Enzymes are biocatalytic proteins. Nearly all reaction steps in living organisms are catalyzed by enzymes. No organism would be able to live without the effect of enzymes; and each reaction step must be catalyzed by a special enzyme. Therefore, enzymes and their functions will be discussed briefly.

Table 3.3 Different groups of proteins and examples for special functions.

Group of proteins	Example	Purpose
Enzymes	Cytochrome	Transfer of electrons in respiration chain
Transport proteins	Haemoglobin	Transport of oxygen in blood
Toxins	Poison produced by yeasts	Repulsion of bacteria
Hormones	Insulin	Regulation of glucose degradation
Structural proteins	Glycoprotein	Components of cell wall

3.2.1.4 Enzymes

The well known Arrhenius equation shows the dependence of the reaction rate on temperature:

$$k = k_o \exp\left(-\frac{E_A}{RT}\right) \tag{3.1}$$

where k is the coefficient of reaction rate, k_o is the theoretical maximal value for $T \rightarrow \infty$ (°C), E_A is the energy of activation (kJ mol^{-1}), R is the general gas constant (kJ mol^{-1} K^{-1}) and T is the temperature (K).

A reduction in e_A leads to an increase in k. The significance of enzymes is their ability to reduce e_A considerably, which results in high reaction rates at moderate temperatures (Fig. 3.5).

The free energy change of a reaction can be measured as the heat of reaction, resulting in a transfer of energy to the surroundings.

The simplest reaction may be the isomerization of a substrate molecule. This can be described in a very simple way by Fig. 3.6a (Scragg 1988).

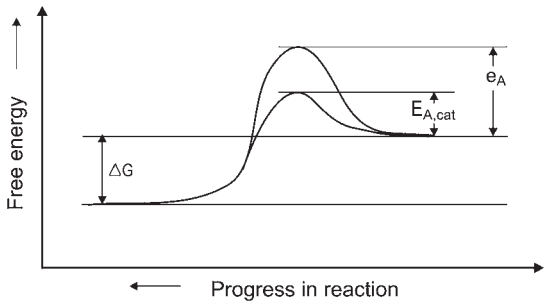

Fig. 3.5 Reduction of energy of activation by an enzyme (catalyst).

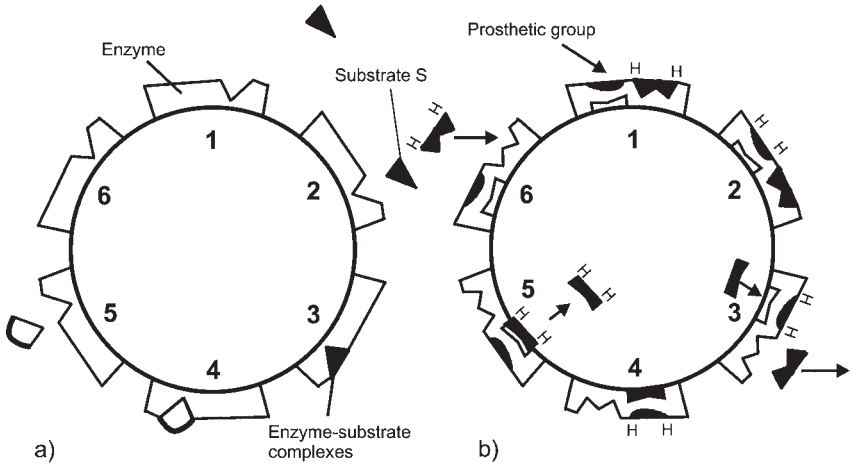

Fig. 3.6 Models for a better understanding for the effects of enzymes. (a) Model for a one enzyme–one substrate reaction without transfer of atoms or molecules (isomerization). (b) Model for a one enzyme–two substrate reaction with the help of a prosthetic group.

The kinetics of this reaction were modelled by Michaelis and Menten (1913), assuming an adsorption equilibrium for the substrate S at the active site of the enzyme (enzyme/substrate complex ES):

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \tag{3.2}$$

A first-order reaction rate is assumed for each step. Writing $r_S = k_3\, S_{ES}$ and $r_{smax} = k_3\, S_{E0}$, we obtain:

$$\frac{r_s}{r_{s\,max}} = \frac{S_{ES}}{S_{E0}} \tag{3.3}$$

and

$$S_{E0} = S_E + S_{ES} \tag{3.4}$$

After a non-stationary period of ES formation, the concentration S_{ES} does not change with time. It is possible to write:

$$\frac{dS_{ES}}{dt} = 0 = k_1 S_E S - k_2 S_{ES} - k_3 S_{ES} \tag{3.5}$$

Considering Eqs. (3.3) to (3.5), the Michaelis–Menten equation follows:

$$r_s = r_{s\,max} \frac{S}{K_m + S} \tag{3.6}$$

with

$$K_m = \frac{k_2 + k_3}{k_1} \tag{3.7}$$

Eq. (3.6) can be transformed easily to linear equations, which are suitable for testing experimental results and for determining $r_{s\,max}$ and K_m (plots called Lineweaver–Burk, Langmuir and Eadie–Hofstee; see Scragg 1988).

A further large group of enzymes called oxidoreductases catalyzes oxidation and reduction reactions (redox reactions) by the transfer of H^+ ions from one molecule to another. Enzymes with a prosthetic group are able to take up two different substrate molecules at two different active sites. The transfer of H^+ progresses via adsorption at the prosthetic group, which does *not* leave the substrate (Fig. 3.6b).

A further system consists of a pair of two different enzymes, each of them for one of two different substrates. Let us discuss this kind of biocatalysis again for the case of a redox reaction. Now the H^+ ions must be transferred from one substrate molecule to the other. For this purpose, a coenzyme is needed as a small part of the substrate molecule which is able to oxidize substrate 1, to leave substrate 1 and to carry the H^+ ions to substrate 2, where substrate 2 is then reduced (Fig. 3.7).

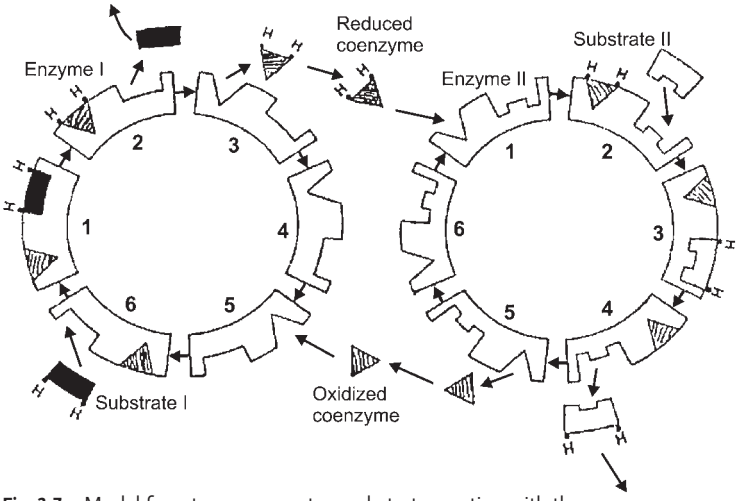

Fig. 3.7 Model for a two enzyme–two substrate reaction with the help of a coenzyme (e.g. redox reaction).

The most important enzymes can be divided into six large groups, which are listed in Table 3.4.

Although a very large amount of information can be stored and transferred by proteins according to their primary structure, proteins exhibit further distinctions forming secondary, tertiary and quaternary structures. Detailed information is given by Cano and Colomé (1986).

Table 3.4 Important groups of enzymes.

Group	Catalyzes the following reaction
Isomerase	Reversible conversion of isomeric compounds
Oxidoreductase	Oxidation and reduction by transfer of hydrogen ions or electrons
Transferase	Transfer of defined groups from one molecule (donor) to another (acceptor)
Hydrolase	Hydrolytic reactions
Lyase	Separation of groups from molecules (without hydrolysis)
Ligase	Combination of two molecules during the splitting of an energy-rich bond

3.2.2 Nucleic Acids

3.2.2.1 Desoxyribonucleic Acid

A nucleic acid is formed from a large number of nucleotides via polymerization. Each nucleotide consists of a heterocyclic nitrogen base, a pentose sugar and a phosphate group (Fig. 3.8a).

Besides adenine as the nitrogen base in Fig. 3.8a, three other bases can be connected to carbon atom 1′ of the pentose molecule. Figure 3.8b shows the four nitrogen bases.

One nucleotide is connected with the next by coupling the marked OH bond (see Fig. 3.9) with the OH bond 3′ on the pentose sugar and releasing H_2O. Each coupled nucleotide is characterized by one of the four heterocyclic nitrogen bases in Fig. 3.8b. In this way, a polynucleotide is formed. Now, a second nucleotide is constructed parallel to the first in the same manner with the opposite base. Cytosine forms a hydrogen bridge with the guanine on the other side and adenine forms the same bond with thymine, its opposite. Both nucleotides spin around each other in such a way that a helix is formed (Fig. 3.9). This helix is turned a second time round the same axis, but with a longer period, forming a double helix (Watson and Crick 1953; Sayre 1992; Wilson 1988).

Figure 3.10 shows the hydrogen bridge in more detail. Thymine and adenine are coupled by two bonds and guanine and cytosine by three bonds, two between O^- and H^+ and one between N^- and H^+.

Hydrogen bridges are relatively weak electrostatic bonds which can be opened by special enzymes during transcription. Because of these hydrogen bridge bonds of differing numbers, it is not possible to form other pairs of bases, especially

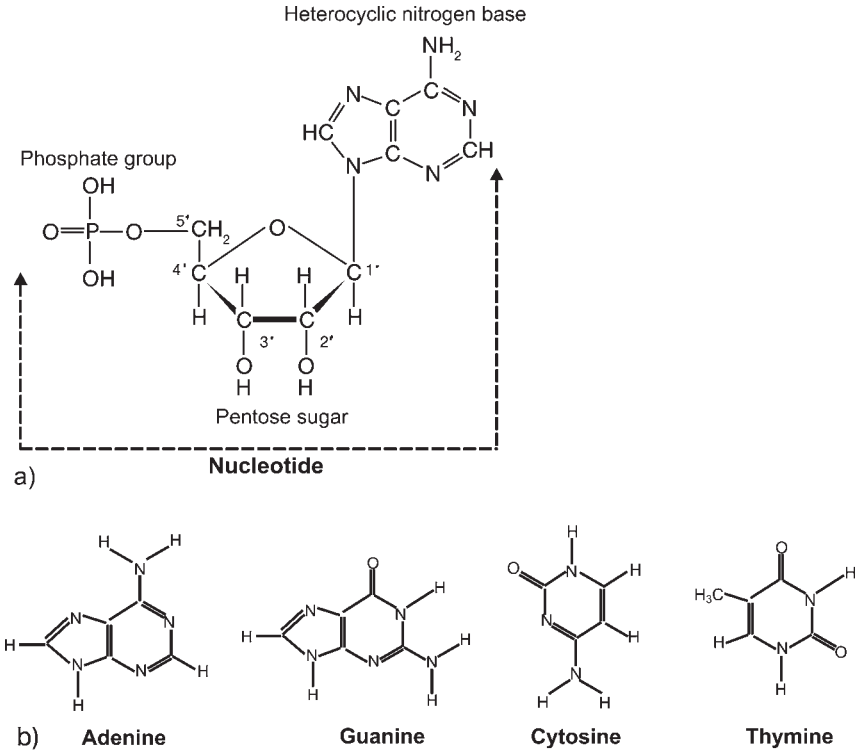

Fig. 3.8 (a) A nucleotide with adenine as one of four different heterocyclic bases. (b) The four heterocyclic nitrogen bases for four different nucleotides forming DNA.

adenine–guanine. Three bases lying side by side are called a triplet. One possible triplet is:

Adenine
Thymine
Guanine
} ATG

The complementary triplet of the opposite side of the deoxyribonucleic acid (DNA) can only be:

Thymine
Adenine
Cytosine
} TAC

Four bases are used for 64 combinations. All of these are used for the genetic code, the building plan of special compounds. The most important compounds are proteins, particularly enzymes. Only 20 amino acids must be produced (Table 3.2). Actually, only 20 triplets would be necessary. Nevertheless, every amino acid is coded by two, three or four triplets. Some amino acids are coded by two different triplets, e.g. phenylalanine by either TTC or TTT. One such set of triplets is called a *codon*

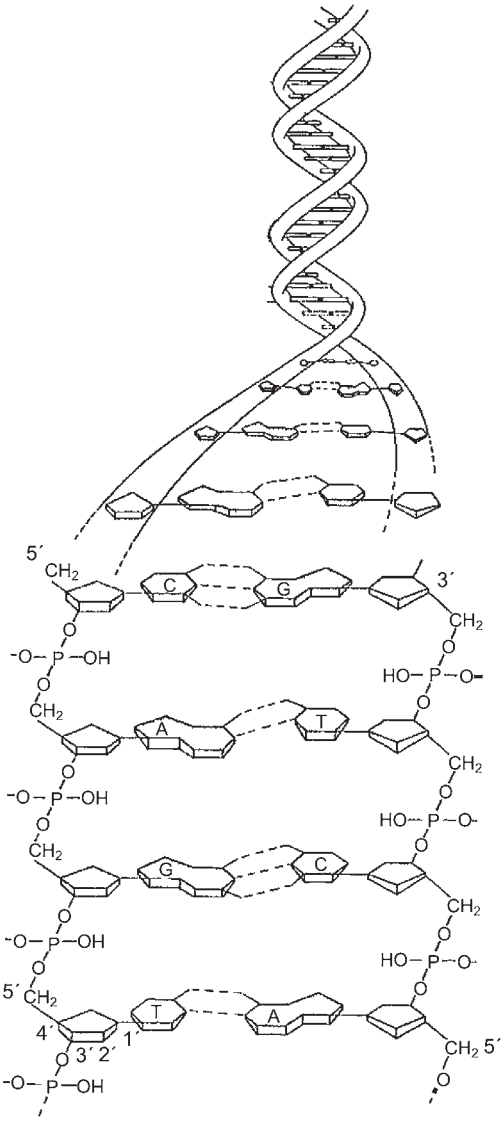

Fig. 3.9 Two polynucleotides coupled by hydrogen bridges forming a DNA molecule in the form of a double helix (Cano and Colomé 1986).

and the opposite side of the DNA the *anticodon*. Several acids are coded by two different codes (Table 3.5).

Three different codes are used for the order "stop" or "termination site". The reading of the building plan for each protein starts and ends with this code.

A section of the DNA which codes the construction of one protein, consisting of four amino acids, could be (see Table 3.5):

TGA	TAT	TAC	AAA	AAG	AGA	AGG	ATG	TGA
stop	Tyr		Lys		Arg		Met	stop

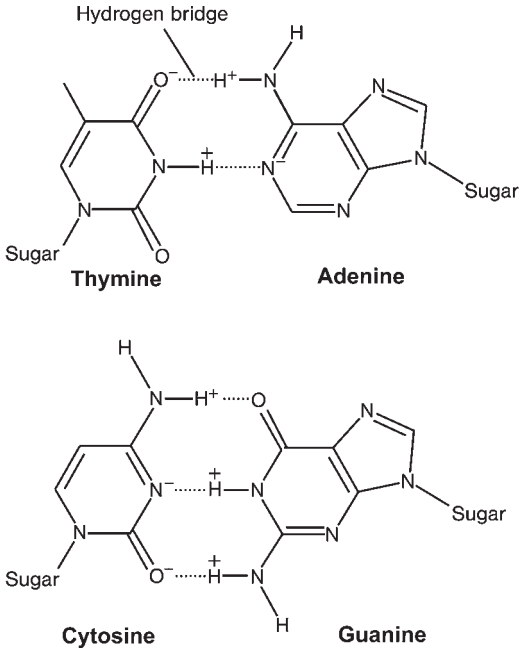

Fig. 3.10 Hydrogen bridges between both base pairs of DNA (Brown 1999).

Table 3.5 The genetic code of amino acids (Brown 1999), written for DNA (instead of RNA).

TTT, TTC: Phe	TCT, TCC, TCA, TCG: Ser	TAT, TAC: Tyr	TGT, TGC: Cys
TTA, TTG: Leu		TAA, TAG: 'Stop'	TGA 'Stop'
			TGG Trp
CTT, CTC, CTA, CTG: Leu	CCT, CCC, CCA, CCG: Pro	CAT, CAC: His	CGT, CGC, CGA, CGG: Arg
		CAA, CAG: Gin	
ATT, ATC, ATA: Ile	ACT, ACC, ACA, ACG: Thr	AAT, AAC: Asn	AGT, AGC: Ser
ATG Met		AAA, AAG: Lys	AGA, AGG: Arg
GTT, GTC, GTA, GTG: Val	GCT, GCC, GCA, GCG: Ala	GAT, GAC: Asp	GGT, GGC, GGA, GGG: Gly
		GAA, GAG: Glu	

3.2.2.2 Ribonucleic Acid

The structure of ribonucleic acid (RNA) is similar to that of DNA. It is also formed by the polymerization of nucleotides. There are, however, three important differences:

- Uracil is used instead of the nitrogen base thymine (Fig. 3.11).
- An RNA molecule consists only of one molecule chain and not two as in DNA.
- RNA molecules are much shorter than DNA molecules.

Figure 3.11 shows the primary structure of an RNA molecule.

We distinguish between three different kinds of RNA:

- Messenger RNA (mRNA).
- Transfer RNA (tRNA).
- Ribosomal RNA (rRNA).

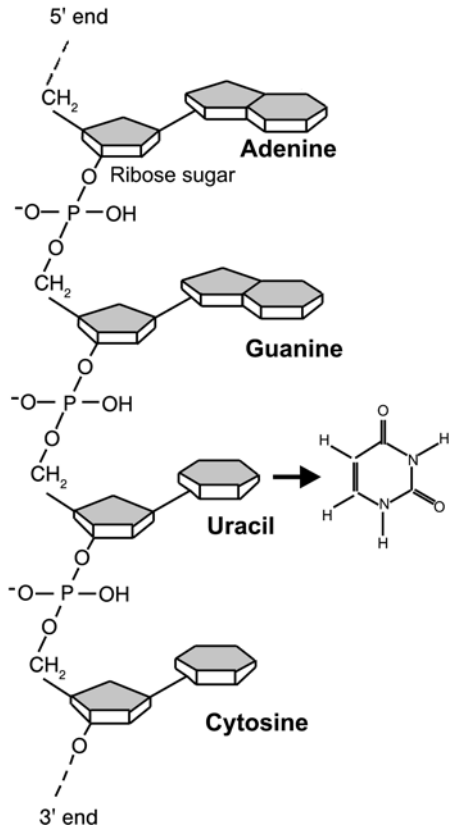

Fig. 3.11 Primary structure of RNA (Cano and Colomé 1986), showing structure of uracil, a nitrogen base in RNA molecules.

mRNA

mRNA arises during the reading of the building plan for a protein which is coded on a section of the DNA, beginning at a triplet coding the promoter side (start) and ending with the termination side (stop; Fig. 3.12a). An enzyme (RNA polymerase) opens both parts of the DNA by cleaving the hydrogen bridges (Fig. 3.12b). Nucleotide molecules diffuse to the complementary nucleotides of the DNA and are coupled together by hydrolysis and reactions at C atoms 3' and 5' of the pentose molecule. The enzyme wanders along the gene, the hydrogen bridges are closed again and the mRNA grows until the termination side is reached (Fig. 3.12c).

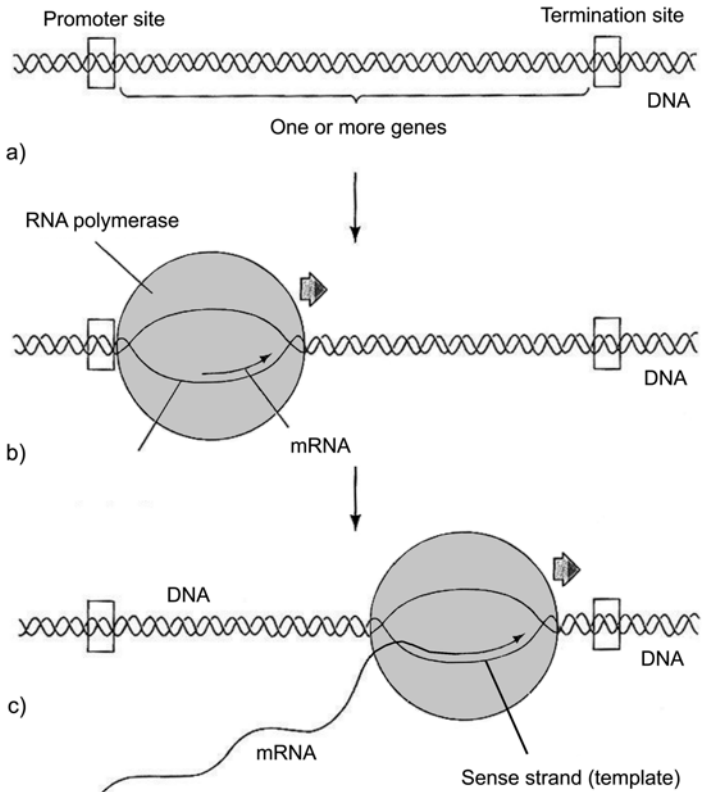

Fig. 3.12 Transcription steps (a–c), forming an mRNA molecule (Cano and Colomé 1986).

tRNA

Compared with mRNAs, which are of different size depending on the size of the coded protein, tRNAs are small polynucleotides and nearly all of the same size. Figure 3.13a gives an impression of the molecule, which is projected in one level. The broken lines show the hydrogen bridges. Figure 3.13b shows that, during reading of the mRNA codon, an anticodon must first be formed which is typical for one amino acid. Thereafter, a second tRNA is joined to the next codon directly left

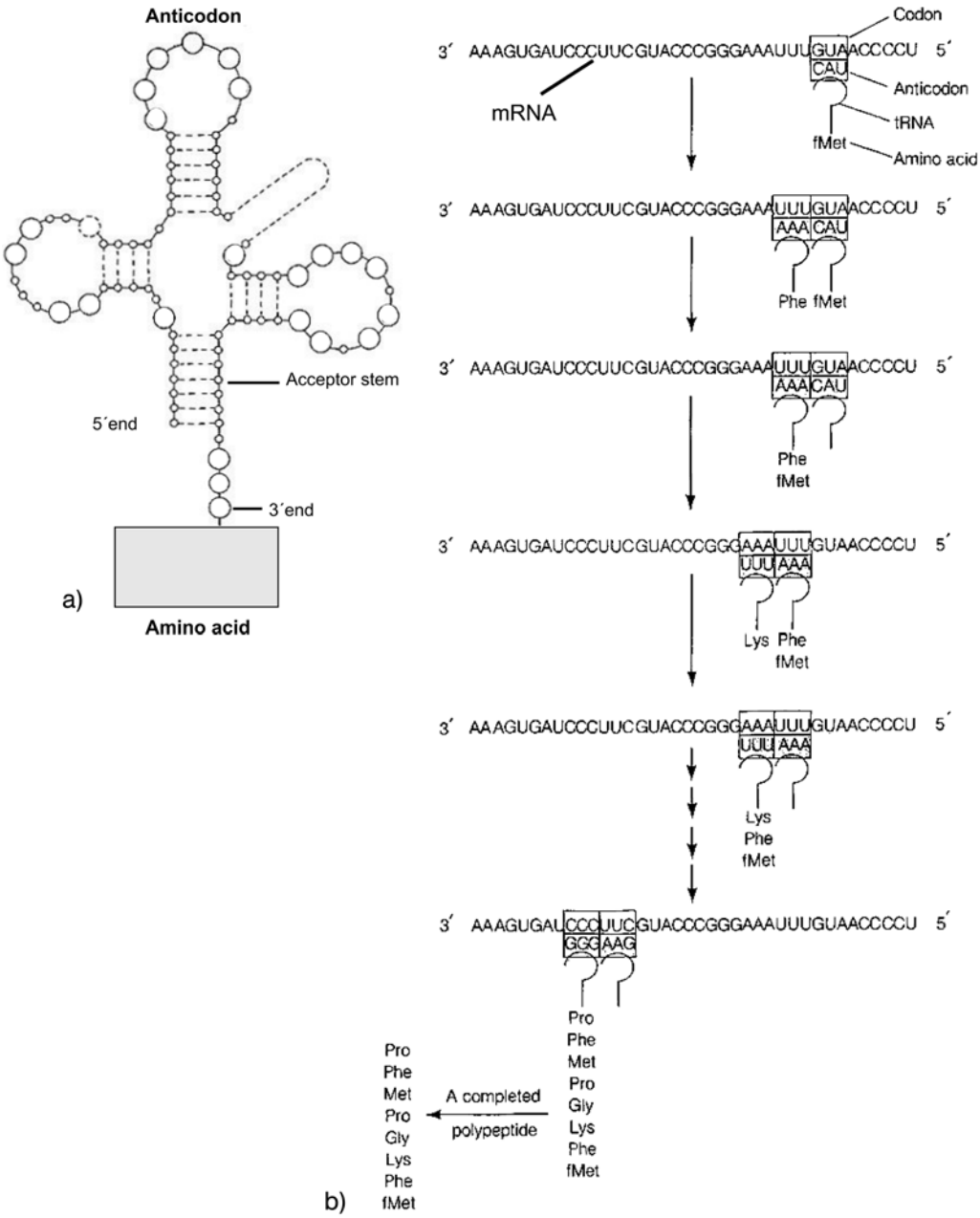

Fig. 3.13 (a) tRNA with anticodon and the typical end for coupling amino acids. (b) Protein synthesis by reading of mRNA (Cano and Colomé 1985).

to the first to obtain its amino acid. In this way, a protein molecule is made which was coded at the section of the DNA being read. The mRNA is used repeatedly for the production of the same protein every time, but after some time it degrades and a new mRNA must be produced. In a similar way, the mRNA is decoded by the rRNA and the according polypeptide grows inside the ribosomes. In ribosomes, the proteins are manufactured. They exist in the cytoplasm. Ribosomal proteins are assembled together with rRNA before being transported through the nuclear membrane. rRNA makes up the vast majority of RNA found in a typical cell. The reading of the mRNA and protein synthesis by tRNA and rRNA are called transmission.

3.2.2.3 **DNA Replication**

During each cell division, a complete copy of the total DNA must be produced, so that both daughter cells obtain the same genes as the mother cell. This process is called DNA replication. Very high precision must be guaranteed. Even one mistake in 10^4 nucleotides may cause changes with serious consequences (Brown 1999).

Here, only the fundamental process of DNA replication will be described shortly. In Fig. 3.14, the opening of the DNA molecule, catalyzed by a special enzyme

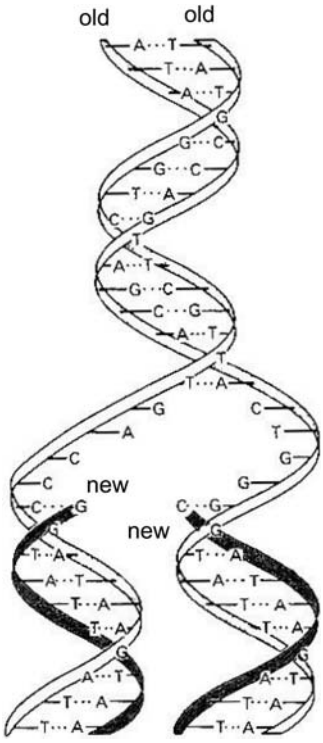

Fig. 3.14 DNA replication after opening the double helix (Brown 1999).

(not shown), is the first step. After that, the nucleotides already synthesized inside the cell are coupled by hydrogen bridges to the complementary base of the mother DNA (thymine and adenine, guanine and cytosine). The last step is the coupling of the two pentose groups (bond 3′ with 5′) via the phosphate group, thereby splitting off one H_2O molecule (Fig. 3.14).

During the division of a bacterial cell, each of the two DNA molecules will be housed in one of the new cells.

3.2.2.4 **Mutations**

Normally DNA replication occurs without any errors. But, considering the enormous number of cells, e.g. such as bacteria, the short time between two consecutive replications and the length of the DNA, with thousands of genes, errors do occur during replication. These are called *mutations*. A mutation is a change in the nucleotide sequence of the DNA during the process of replication.

We distinguish several kinds of mutations:

- Transition
  - A is replaced by G
  - T is replaced by C
- Transversion
  - A is changed to T
  - T is changed to A
- Addition
  - A is replaced by A C
  - T is replaced by T G
- Deletion
  - A is deleted
  - T is deleted
- Inversion
  - AAA CCC is inverted to GGG TTT
  - TTT GGG is inverted to CCC AAA

In all these cases, one of the two daughter cells has DNA which is not the same as the mother DNA and it may exhibit some new properties.

The rate of mutations can be influenced by chemical reactions between special compounds and DNA. Further, the rate of these reactions is influenced by pH, temperature, concentration and other physical parameters, such as the intensity of UV radiation. These mutations make it possible for microorganisms to degrade new organic substances produced synthetically in the chemical industry (chlorinated benzenes and phenols, dyes, etc.), which never occurred in nature before.

3.3
Catabolism and Anabolism

3.3.1
ADP and ATP

All the processes of synthesis described above need energy, which is provided by catabolic reactions. During these catabolic reactions, chemical energy is stored by forming ATP from a compound with lower energy, ADP; and it is then used partly as chemical energy and is partly converted into thermal energy, resulting in an increase in temperature.

The structures of ADP and ATP are given in Fig. 3.15.

To store energy, a molecule of phosphoric acid (H_3PO_4) is coupled to ADP, forming ATP with the help of enzyme A:

$$\mathrm{ADP + H_3PO_4 + 29.97\ kJ\ mol^{-1} \underset{\text{enzyme B}}{\overset{\text{enzyme A}}{\rightleftharpoons}} ATP + H_2O} \qquad (3.8)$$

According to that chemical balance, the same energy is available at a later time when enzyme B is engaged.

There are three mechanisms for the coupling and decoupling of a phosphoric acid group (phosphorylation). The most important for the catabolic pathways discussed later is called substrate-level phosphorylation.

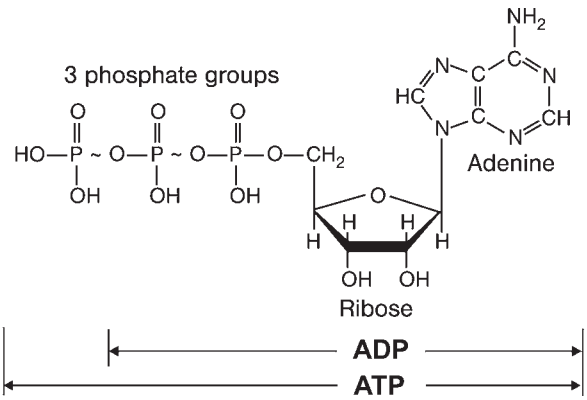

Fig. 3.15 Adenosinediphosphate (ADP) and Adenosinetriphosphate (ATP).

3.3.2
Transport of Protons

The catalytic effect of enzymes is often combined with a transport of protons or H^+ by a coenzyme. This was already explained in Section 3.2.1.4 (Fig. 3.6a, b). Two of the most important coenzymes are nicotine amide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD). Figure 3.16a shows NAD in the oxidized

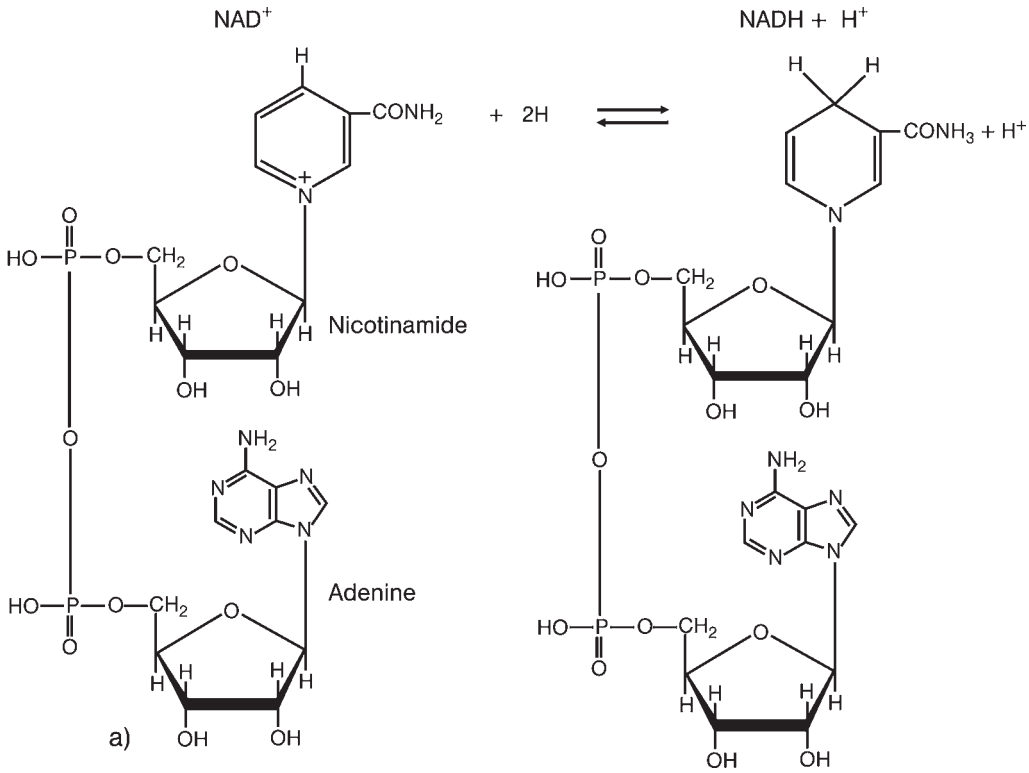

Fig. 3.16 (a) Equilibrium between NAD^+ and $NADH + H^+$ (Cano and Colomé 1986, modified).

form NAD^+ and in the reduced form $NADH + H^+$ ($NADH_2$); and Fig. 3.16b shows the coenzyme FAD.

Positively charged NAD^+ loses its charge during reduction and is combined with a proton ($NADH + H^+$). This is only possible by reorientation of the double bonds and reduction of the five-valent nitrogen atom of the nicotine amide group to a three-valent one. The FAD molecule is constructed similarly (Fig. 3.16b). The $NADH_2$ coenzyme diffuses to the same enzyme (see Fig. 3.6a) or to another enzyme (see Fig. 3.6b), where another substrate is to be reduced. The importance of NAD and FAD will be discussed in the following catabolic degradation of glucose.

3.3.3 Catabolism of Using Glucose

3.3.3.1 Aerobic Conversion by Prokaryotic Cells

Most prokaryotic cells (bacteria) and all eukaryotic organisms obtain their energy from *chemoorgano heterotrophic* metabolism. Part of this energy is stored in ATP molecules and part of it is transformed into heat. The mechanism of ATP produc-

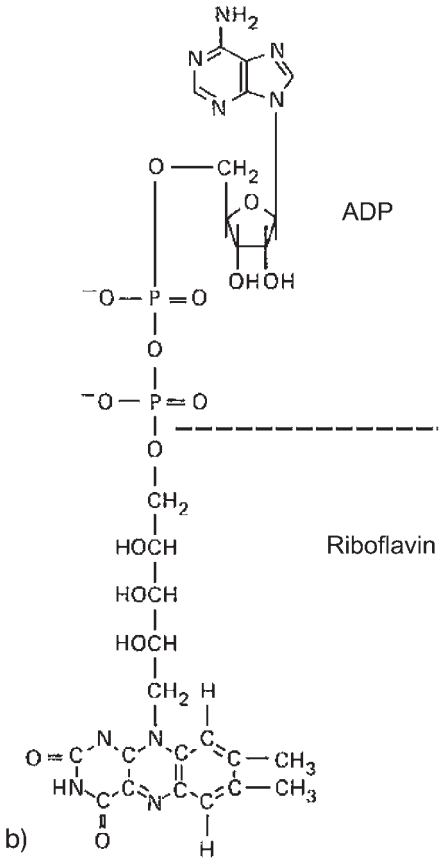

Fig. 3.16 (b) Structure of FAD (Scragg 1988).

tion is normally described using glucose as an example; and it is divided into three stages:

- the glycolysis or Embden–Meyerhoff pathway,
- the citric acid cycle,
- the respiration chain.

During the first part of glycolysis (Fig. 3.17) two ATPs must be reduced to two ADPs. From one glucose molecule (6 C), two molecules of glycerine aldehyde-3-phosphate (3 C) are produced.

Therefore, the pathway to the end-product of glycolysis pyruvate must be travelled two times, producing 2 $NADH_2$ and 2 ATP:

$$\begin{aligned} &C_6H_{12}O_6 + 2\ H_3PO_4 + 2\ ADP + 2\ NAD \rightarrow \\ &2\ CH_3COCOOH + \boxed{2\ NADH_2} + \boxed{2\ ATP} + 2\ H_2O \end{aligned} \tag{3.9}$$

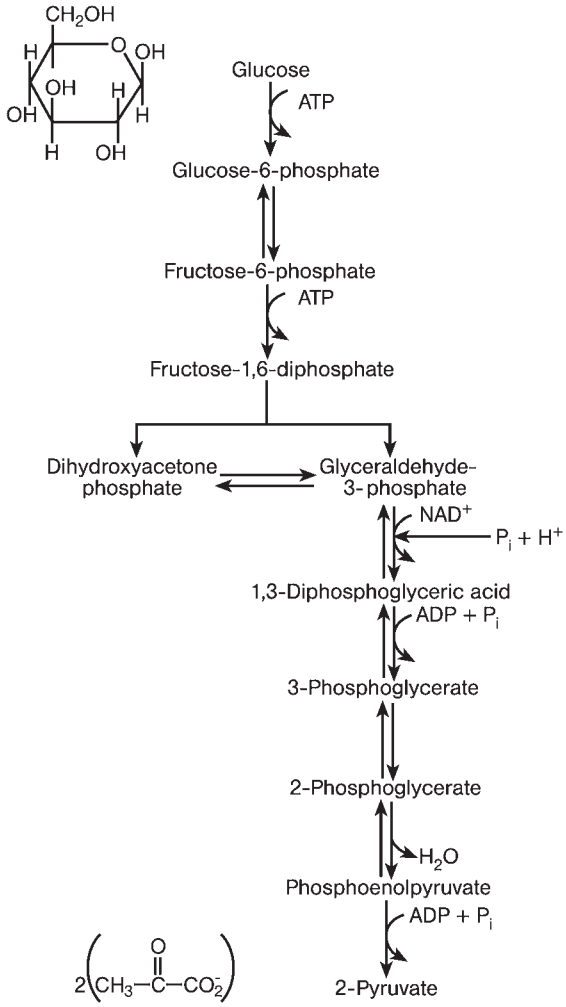

Fig. 3.17 Glycolysis (Cano and Colomé 1986).

Two pyruvates are now mineralized by the nine steps of the citric acid cycle, after being oxidized by NAD and coupled to coenzyme acetyl CoA (Fig. 3.18):

$$2\ CH_3COCOOH + 2\ NAD + 2\ CoAH \rightarrow$$
$$2\ CH_3CO\text{-}CoA + \boxed{2\ NADH_2} + 2\ CO_2 \qquad (3.10)$$

Only with the help of this coenzyme is it possible for active acetic acid to enter the citric acid cycle and react with oxaloacetic acid (4 C), producing citric acid (6 C). Note that the cycle is passed through two times, producing 2 GTP, 6 $NADH_2$ and 2 $FADH_2$, and that CoA is reduced to CoAH again. It is used repeatedly (see Eq. 3.10) and produced during the two steps from α-ketoglutaric acid to succinic

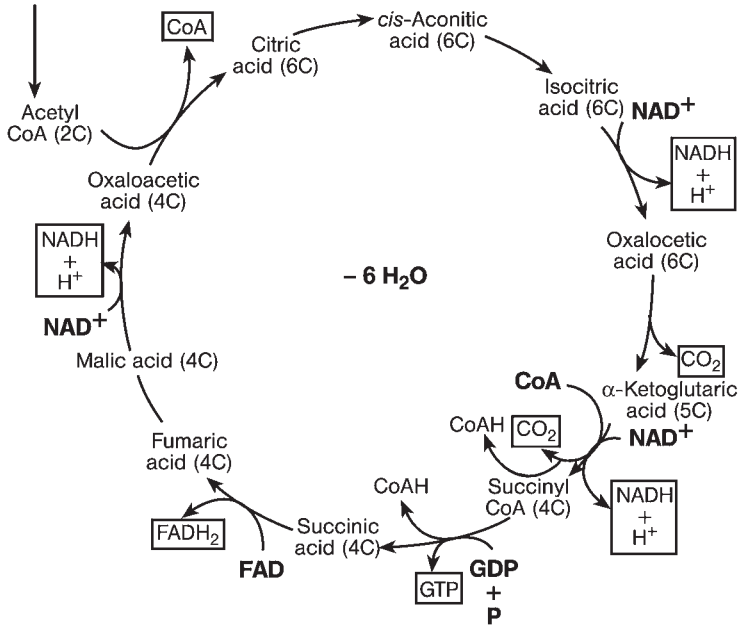

Fig. 3.18 Citric acid cycle (Cano and Colomé 1986).

acid. GDP is constructed similarly to ATP; and thus we write ATP instead of GTP. The balance for the complete citric acid cycle is:

$$\begin{aligned} &2\ CH_3CO\text{-}CoA + 6\ H_2O + 6\ NAD + 2\ FAD + 2\ ADP + 2\ H_3PO_4 \rightarrow \\ &2\ CoAH + \boxed{6\ NADH_2} + \boxed{2\ FADH_2} + \boxed{2\ ATP} + 2\ H_2O + 4\ CO_2 \end{aligned} \tag{3.11}$$

Note that: $2\ ADP + 2\ H_3PO_4 \leftrightarrow 2\ ATP + 2\ H_2O$ and $FAD^+ + 2H \rightarrow FADH + H^+$ ($FADH + H^+ = FADH_2$).

Altogether, $10\ NADH_2 + 2\ FADH_2$ are now oxidized, passing now eight links of the respiration chain or electron transport system. Figure 3.19 presents a qualitative plot of the energy level versus the course of the reaction. If $NADH_2$ is oxidized, the hydrogen atom is transported from the first link to the last and oxidized by O_2 to H_2O.

The chemical energy is used to convert 3 ADP to 3 ATP (second and fourth links). The chemical energy of $FADH_2$ is lower by about one-third, resulting in only two ADPs after the uptake by coenzyme Q (third link). The balance of the electron transfer chain can be written as follows:

$$\begin{aligned} &10\ NADH_2 + 2\ FADH_2 + 34\ ADP + 6\ O_2 \rightarrow \\ &10\ NAD + 2\ FAD + 34\ ATP + 46\ H_2O \end{aligned} \tag{3.12}$$

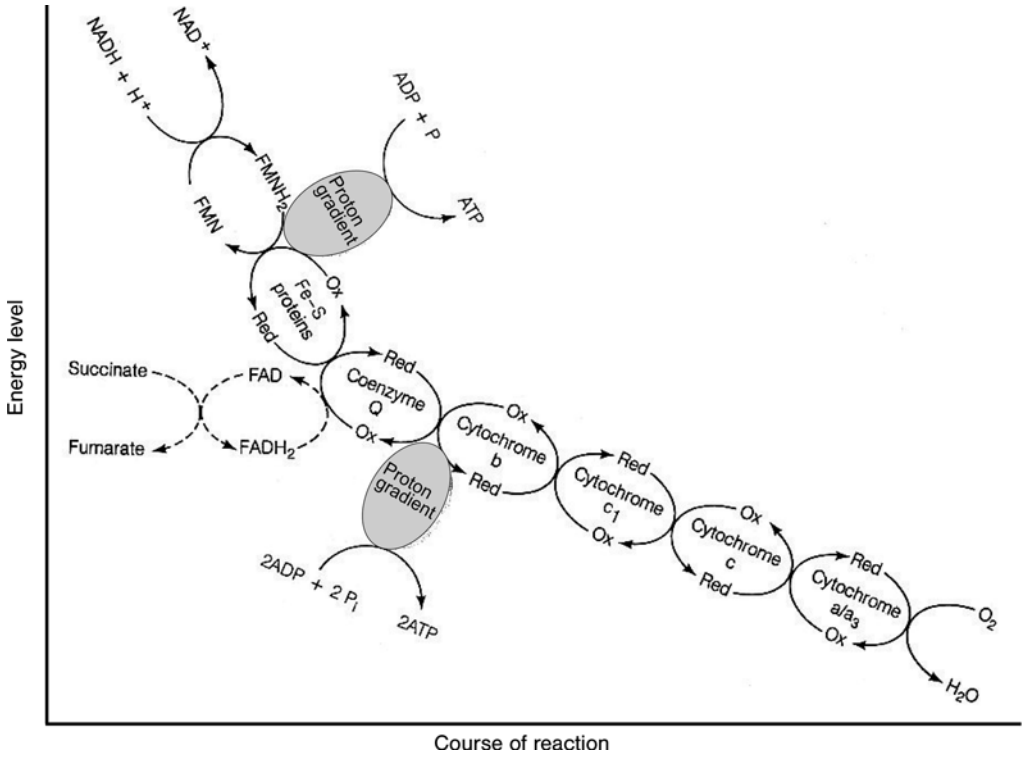

Fig. 3.19 Electron transport system of respiration chain (Cano and Colomé 1986).

Remember that: 34 ADP + 34 $H_3PO_4 \leftrightarrow$ 34 ATP + 34 H_2O.

Alltogether, 2 ATP (Glycolysis) + 2 ATP (Citric acid cycle) + 34 ATP = 38 ATP are produced.

If we summarize Eqs. (3.9) to (3.12), we obtain the known balance for the aerobic catabolic degradation of glucose:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 2826\ \text{kJ mol}^{-1} \tag{3.13}$$

In prokaryotic cells (bacteria), a part of this relatively high amount of energy is stored as ATP, namely 29.97 kJ mol^{-1} per ATP. Therefore, the efficiency of this energy storage is:

$$\eta = \frac{38 \cdot 29.97}{2826} = 0.403 \text{ or } 40.3\%$$

The rest, or nearly 60%, is converted into thermal energy.

3.3.3.2 Anaerobic Conversion by Prokaryotic Cells

If we simply replace oxygen with inorganic electron acceptors (NO_3^-, NO_2^-, SO_4^{2-}) or with hydrogen, catabolism is nearly the same as in the aerobic case, with the exception of the respiration chain. Therefore, a lower amount of ATP is produced. Environmental scientists call this *anoxic* metabolism. We will come back to this topic, when we discuss denitrification in Chapter 10.

Anaerobic metabolism (Zehnder 1988) is characterized by a transfer of electrons or hydrogen to organic compounds, resulting in organic products which cannot be used further by the same bacteria. This process, which is at the center of interest for biotechnologists, is called *fermentation*. For this reason, bioreactors are called *fermenters*. Unfortunately, biotechnologists and microbiologists use the term fermenter even if aerobic or anoxic reactions are taking place.

Here, two examples will be discussed briefly:

Production of Lactic Acid

Bacteria which produce lactic acid (e.g. *Lactobacillus bulgaricus*) are of great importance for the food industry. Because of their relative insensitivity with regard to low pH, they are used for food sterilization (yoghurt, sauerkraut, pickling, etc.). As the bacteria produce more and more lactic acid and the concentration rises, the pH falls and increasing numbers of bacteria are killed off, until even the lactobacilli themselves die. *L. bulgaricus,* for example, is used to produce buttermilk.

The first stage of catabolism is the glycolysis or Embden–Meyerhoff pathway (Fig. 3.17) if glucose is used. The balance is given by Eq. (3.9). Pyruvate is not coupled to coenzyme CoAH as shown by Eq. (3.10), rather it is reduced directly by $NADH_2$ to lactic acid:

$$2\ CH_3COCOOH + 2\ NADH \rightarrow 2\ CH_3CHOHCOOH + 2\ NAD \tag{3.14}$$

Summarizing Eqs. (3.9) and (3.14), we obtain:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH + 2\ H_2O \tag{3.15}$$

and

$$2\ H_3PO_4 + 2\ ADP \rightarrow 2\ ATP + 2\ H_2O \tag{3.16}$$

The greatest part of the chemical energy of 1 mol glucose remains in 2 mol lactic acid, resulting in only 2 mol ATP and a small increase in biomass.

Production of Ethanol

Yeasts functioning as eukaryotic cells (e.g. *Sacharomyces cerevisiae*) and bacteria (e.g. *Zymomonas mobilis*) produce ethanol as their end-product of catabolism. After glycolysis, pyruvate is transformed into acetaldehyde and CO_2:

$$C_6H_{12}O_6 + 2\ NAD \rightarrow 2\ CH_3CHO + 2\ CO_2 + 2\ NADH_2 \tag{3.17}$$

Acetaldehyde is reduced by $NADH_2$ to ethanol in the final step:

$$2\ CH_3CHO + 2\ NADH_2 \rightarrow 2\ CH_3CH_2OH + 2\ NAD \quad (3.18)$$

Summarizing Eqs. (3.17) and (3.18), we obtain:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2 \quad (3.19)$$

(Zehnder 1988) and:

$$\Delta G^\circ = -244.9\ kJ\ mol^{-1}$$

Because fermentation to ethanol is inhibited by the end-product, only about 18vol% ethanol is attainable (wine, beer, etc.). For more highly concentrated alcoholic drinks, water must be removed by distillation and condensation or by reverse osmosis. Ethanol can also be produced from liquid wastes, which are predominately polluted with mono-, di- and polysaccharides, or renewable raw materials, and then used as a fuel additive to gasoline.

In anaerobic wastewater treatment processes, acetic acid is produced by the fermentation of higher fatty acids. This is discussed in more detail in Chapter 8.

3.3.4 Anabolism

Bacteria are characterized by a remarkable achievement during their growth. *E. coli*, for example, synthesizes 1400 protein molecules in 1 s. With 300 peptide bonds in each molecule, this makes 420 000 bonds s^{-1}. To reach this rate of protein synthesis, 88% of the energy stored in ATP is needed. Since 6 ATP are used for one peptide bond, $2.5 \cdot 10^6$ ATP must be used every second! The energy stored in an *E. coli* cell is sufficient for only 2 s, with its ATP content of $5.0 \cdot 10^6$ ATP.

Table 3.6 gives an impression of the work rate of *E. coli* biosynthesis per second.

This extremely high capacity can only be realized by very economical synthetic reactions. Therefore, substrates which contain proteins are only hydrolyzed to amino acids, which are then coupled together with new proteins. Only by these and other optimizations are bacteria able to achieve extremely economical anabolism.

Table 3.6 Biosynthesis capacity of *E. coli* during a 20-min cell division (Lehninger 1965).

Chemical component	Dry weight (%)	Approximate molar mass	Number of molecules per cell	Number of molecules synthesized per second	Number of molecules of ATP synthesized per second	Total biosynthetic energy required (%)
DNA	5	$2 \cdot 10^9$	1	0.00083	$60 \cdot 10^3$	2.5
RNA	10	$1 \cdot 10^6$	$15 \cdot 10^3$	12.5	$75 \cdot 10^3$	3.1
Protein	70	$60 \cdot 10^3$	$1.7 \cdot 10^6$	1400	$2.12 \cdot 10^6$	88.0
Lipid	10	$1 \cdot 10^3$	$15 \cdot 10^6$	12500	$87.5 \cdot 10^3$	3.7
Polysaccaride	5	$2 \cdot 10^5$	$39 \cdot 10^3$	32.5	$65 \cdot 10^3$	2.7

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