Structure and Functions of Microorganisms: Production and Use of Material and Energy

3

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Abstract

The cellular structures of prokaryotic and eukaryotic microorganisms and the characters distinguishing the three domains of life (*Archaea*, *Bacteria*, *Eukarya*) are first described. Then, the metabolic diversity of microorganisms is discussed, the knowledge of which is essential to understand the role of microorganisms in natural and anthropogenic environments. The different degradation pathways for mineral and organic compounds that provide cellular energy are described (aerobic and anaerobic respirations, fermentations) as well as the photosynthetic processes (aerobic and anaerobic photosynthesis). Finally, the mechanisms of biosynthesis are presented: autotrophy and heterotrophy, assimilation of C1, and assimilation of organic and inorganic compounds (mainly nitrogen and sulfur assimilation).

Keywords

Aerobic respirations • Anaerobic respirations • CO_2 assimilation • Eukaryotic cells • Fermentations • Heterotrophic biosynthesis • Inorganic compound assimilation • Photosynthesis • Prokaryotic cells

3.1 Structure and Functions of Prokaryotes and Eukaryotes: Major Features and Differences

The living world is now divided into three major areas (see the second part of the book, Chaps. 5, 6, and 7) in which the cellular structure is either of the prokaryotic or eukaryotic type. The *Bacteria* and *Archaea* domains consist of microorganisms of usually unicellular prokaryotic type, and the *Eukarya* domain includes microorganisms and multicellular organisms that are all of eukaryotic type. One major difference between prokaryotic and eukaryotic cells is the absence of a nuclear membrane in prokaryotes which is needed to define a true nucleus (chromosomes have been separated from the cytoplasm by the nuclear membrane) in eukaryotes (from the Greek *eu*: true, *caryon*: nucleus). In prokaryotes, the generally circular chromosome present in a single copy is directly in the cytoplasm (pro: that precedes, *caryon*: nucleus). In some prokaryotes, it may be linear and in multiple copies (*Rhodobacter sphaeroides*: two copies; *Halobacterium* sp.: three copies). Both types of structure are also differentiated by their size and cell contents including the presence of various organelles in eukaryotes (Fig. 3.1a; Table 3.1).

3.1.1 Prokaryotic Microorganisms (*Bacteria* and *Archaea*, *cf.* Chaps. 5 and 6)

Prokaryotes are unicellular microorganisms. However, some may associate to form clusters more or less regular, single filaments or branched filaments from a few cells to hundreds

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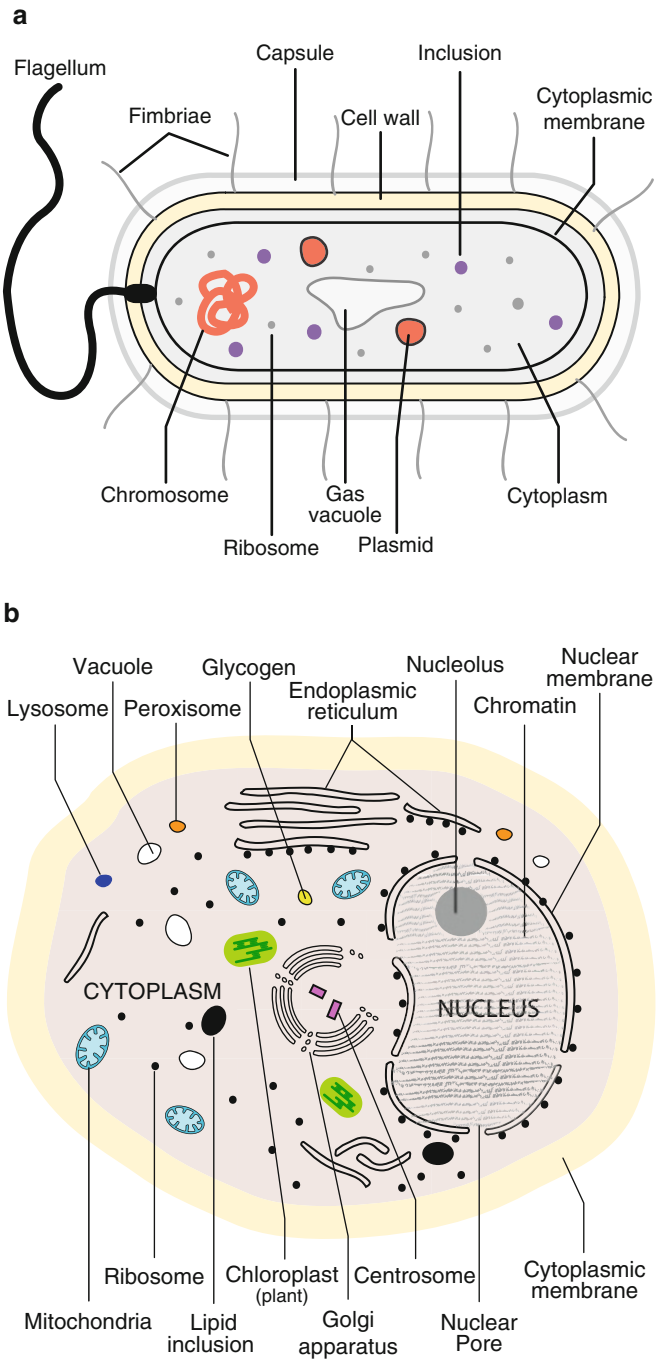

Fig. 3.1 Schematic representation of prokaryotic (**a**) and eukaryotic (**b**) cells (Drawing: M.-J. Bodiou)

of cells, some with functional specificity, the first step toward multicellularity (*cf.* Sect. 5.2). The cells of prokaryotes are generally small, close to the micrometer, spherical (cocci) or elongated rod shaped, straight, curved, or spiral. Particular forms have stalks or protuberances (Fig. 3.2). Some prokaryotic cells may have large sizes, visible with the naked eye (such as *Thiomargarita namibiensis*, 300–750 µm in diameter) (*cf.* Chap. 5, Fig. 5.1c); in contrast, nanobacteria whose cells are less than 0.2 µm in diameter were isolated from different environments.

Exchanges between cells and the external environment are realized at the cell surface. The surface/volume ratio which is inversely proportional to cell size gives an advantage to prokaryotes compared to all other living organisms. However, this feature makes it more sensitive to changes in the environment. Whatever the form, prokaryotic cell organization remains the same: more or less complex cell envelopes are surrounding cytoplasm that contains a small number of inclusions (chromosome, plasmids, ribosomes, gas vacuoles, etc.). Some also contain a membrane

Table 3.1 Some characteristics of differentiation between the three domains of life

	Bacteria	*Archaea*	*Eukarya*
Organelles	Absent	Absent	Present
Nuclear membrane	Absent	Absent	Present
Cellular wall	Present muramic acid present	Present muramic acid absent	Present or absent
Membrane lipids	Linear chains	Branched aliphatic chains	Linear chains
Circular DNA	Present	Present	Absent
Introns	Absent	Absent	Present
Operons	Present	Present	Rare
mRNAs			
RNA polymerases	One	Several	Three
TATA sequence in the promoter	Absent	Present	Present
Addition of a polyA sequence	Rare	No	Yes
Addition of methylguanosine	No	No	Yes
Transfer RNA			
Thymine in the T Ψ C loop	In general present	Absent	In general present
Dihydrouracil	In general present	In general absent	In general present
AA carried by initiator tRNA	Formylmethionine	Methionine	Methionine
Ribosomes			
Size in Svedberg units	70 S	70 S	80 S
Size of two subunits in Svedberg units	30 S, 50 S	30 S, 50 S	40 S, 60 S
Size of RNA in Svedberg units	16 S	16 S	18 S
Functions			
Anaerobic respirations	Present	Present	Generally absent
Methanogenesis	Absent	Present	Absent
Chemolithotrophy	Present	Present	Absent
Chlorophyll photosynthesis	Present	Absent	Present

defining an inner cytoplasmic zone containing the chromosome (*cf.* Sect. 6.6.2: phylum *Planctomycetes*).

3.1.1.1 Cell Envelopes of Prokaryotes

Prokaryotic cells have generally two types of envelopes; on the outside, the rigid wall maintains the shape of the cells and covers the cytoplasmic membrane delimiting the cytoplasm. The composition of the wall defines three groups of prokaryotes: Gram-positive bacteria (Gram +), Gram-negative bacteria (Gram−), and archaea. *Mycoplasma* and some archaea do not have a wall.

Cytoplasmic Membrane

In bacteria, the cytoplasmic membrane is composed of proteins (*cf.* Sect. 4.1.7, Fig. 4.5) and a phospholipid bilayer (Fig. 3.3) that forms the basic structure. In this double layer, phospholipids are oriented such that the apolar chains are placed inside and polar ends located on the surface of the membrane. Many molecules are included in the membrane:

1. Terpenoid derivatives (hopanes) acting as stabilizers of membrane structure (*cf.* Sect. 4.1.5, Fig. 4.15; *cf.* Sect. 16.8.2, Fig. 16.40).
2. Enzymes, pigments, and electron carriers involved in respiratory and photosynthesis activities. The cytoplasmic membrane of prokaryotes is the principal location of the production of cellular energy. This energy is derived from enzymes, pigments, and electron carriers involved in the respiratory and bacterial membrane proton-motive force (Δp) resulting from the formation of a transmembrane proton gradient (*cf.* Sect. 3.3.1).

In archaea, the cytoplasmic membrane plays the same role, but its bilayer or monolayer structure is different. Branched hydrocarbon chains associated with the glycerol by ether linkages – stronger than the ester linkages of bacteria – replace linear fatty acids. Branched chains have variable lengths in carbon number of C20 (bilayer) to C40 (monolayer). In the latter case, typical of many extreme thermophilic archaea, the monolayer consists of molecules having two glycerols linked by four ether linkages (tetraether) to the ends of the two branched chains, allowing for greater stability and rigidity of the membrane at high temperatures (*cf.* Sect. 4.1.7). Diethers and tetraethers may be mixed with lipids (phospholipids, sulfolipids, or glycolipids). The *Halobacteria* (extreme halophilic archaea) synthesize a modified membrane, the purple membrane, by inserting of a protein pigment, bacteriorhodopsin, close to the rhodopsin of the retina of the eye. The activation of this pigment by light allows the membrane energy production via the formation of a gradient of protons (*cf.* Sect. 3.3.4, Fig. 3.30).

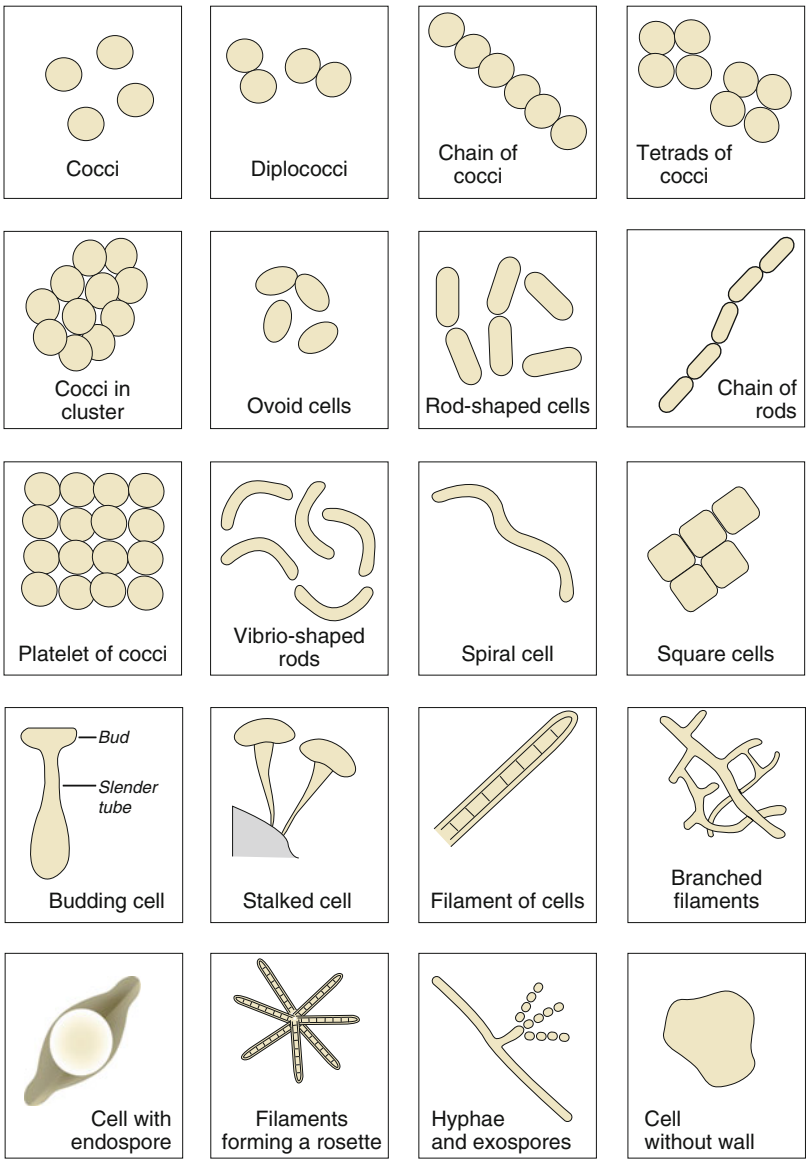

Fig. 3.2 Some shapes and associations of prokaryotic cells (Drawing: M.-J. Bodiou)

The membrane structure is a fluid mosaic in which phospholipids move quickly in their own layer and slowly from one layer to the other resulting in the displacement of other molecules. The membrane is a diffusion barrier that controls the exchanges of ions, solutes, metabolites, or macromolecules between the inside of the cell and its environment. The transport of substances through the membrane interacts with the cellular metabolism.

Simple diffusion transports concern small inorganic or organic molecules (O_2, CO_2, NH_3, H_2O, ethanol, etc.). They require a concentration gradient on both sides of the membrane and do not allow the intracellular accumulation of substances (passive diffusion). However, specific proteins may help the passage of solutes in the presence of a concentration gradient; it is the facilitated diffusion that does not involve energy and allows passage of molecules such as glycerol, fatty acids, aromatic acids, etc.

Active transports are used to transport substances when they are in low concentration in the environment of the cell. These transports, allowing accumulation of substances against concentration gradients, require energy. Several mechanisms are involved:

1. ABC transporters (ATP-binding cassette transporters) use the binding proteins located on the external face of the membrane that bind to the substance to be transported (mono- or disaccharides, amino acids, organic acids, nucleosides, some inorganic ions). These proteins then bind to an intramembranous specific carrier. Finally, energy from the hydrolysis of ATP releases the nutrient that is transferred into the cytoplasm.

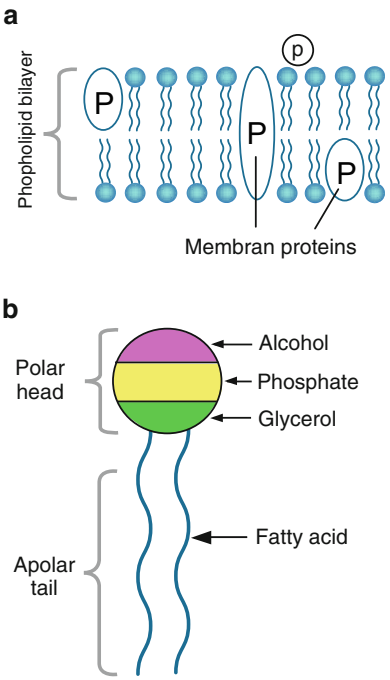

Fig. 3.3 The cytoplasmic membrane, general organization. (**a**) General scheme of the cytoplasmic membrane. (**b**) Schematic organization of a phospholipid (alcohols = glycerol, ethanolamine, etc.) (Drawing: M.-J. Bodiou)

2. Other transporters, secondary active transporters, use the energy of a proton or concentration gradient. Some are symport transporters for which penetration of the substance (sugars, amino acids) is generally associated with a positive gradient of protons or Na^+, the transfer of protons or Na^+ resulting in the passage of the active substance (Fig. 3.4). Other carriers are antiport transporters in which the transport of the substance is associated with a negative gradient (against gradient) of Na^+ or proton or of another substance, for example, the penetration of malate is associated with the output of lactate during the malolactic fermentation in lactobacilli.
3. During active transport, the transported molecules can be chemically modified. The best known mechanism is the transport of sugars (glucose, fructose, mannose) involving the phosphotransferase system which phosphorylates the sugars at the level of carrier and releases the phosphorylated sugar in the cytoplasm; for example, in the case of glucose transport, the phosphate group of glucose-6-phosphate originates from the hydrolysis of the phosphate bond of phosphoenolpyruvate.

In phototrophic purple bacteria, the cytoplasmic membrane, which is folded on itself, is at the origin of complex structures in the form of tubules, lamellae, folds, or vesicles according to bacterial groups (Fig. 3.27). These invaginations, observed also in nitrifying bacteria, increase the surface-to-cell volume ratio and thus promote exchange and energy activities.

Cell Wall

The cell wall allows the maintenance of cell shape; it is less complex in Gram-positive than in Gram-negative bacteria.

In bacteria staining Gram positive, the wall is made as a thick and rigid peptidoglycan or murein (Fig. 3.5b). Peptidoglycan which represents 90 % of weight of the wall is a very large polymer formed by the sequence of two derivatives of amino sugars, N-acetylglucosamine (AG) and N-acetylmuramic acid (AM). Residues of AM are substituted by tetrapeptides (TP). Polypeptide bridges (e.g., pentaglycine bridge) connect the tetrapeptides between them and thus form a rigid entanglement (Fig. 3.5a). Teichoic acids consisting of phosphoglycerol or ribitol phosphate polymers, which are sometimes associated with sugars and alanine, are also involved in wall rigidity.

In bacteria staining Gram negative, the wall structure is more complex (Fig. 3.5b). The peptidoglycan is thinner and represents only 10 % of the wall; there is no teichoic acid. On the external side, the wall is limited by a membrane, the outer membrane (Fig. 3.5c). The outer layer of this membrane contains chains of lipopolysaccharides (LPS) which extend outside the membrane forming side chains (antigen O). The LPS is the endotoxin of Gram-negative bacteria. The outer membrane is a diffusion barrier less effective than the cytoplasmic membrane. Proteins form channels (porins) for non-specific passage of small molecules. The larger molecules are transported specifically through the membrane. The space between the outer membrane and cytoplasmic membrane is called the periplasmic space or periplasm. It contains many enzymatic proteins and may represent up to 40 % of bacterial volume (Fig. 3.5b). Some Gram-negative bacteria secrete into the environment outer membrane nanovesicles formed by the bacterial outer membrane and containing components of the periplasm and cytoplasm (enzymes and toxins).

In archaea, the cell wall has a different composition and is more variable. Some archaea have walls with a single thick layer formed of various polymers such as pseudomurein in which N-acetylgalactosamine replaces N-acetylglucosamine. Others have walls consisting of polysaccharides or heteropolysaccharides which are sulfated or non-sulfated. The wall of other archaea is formed of a layer or double layer of protein or glycoprotein subunits (Madigan et al. 2010).

Some bacteria and archaea are devoid of wall; in this case, the cytoplasmic membrane is the unique cellular envelope.

The prokaryotic microorganisms are frequently surrounded by a mucous layer called the glycocalyx. This layer is composed of polysaccharides which are often associated with polypeptides and that are secreted and accumulated around the cell. The glycocalyx may be a thin mucous layer called EPS ("exopolysaccharides" or "exopolymeric substances") or a thick layer more or less rigid, called the capsule. These highly hydrated exopolymers allow cells to agglomerate into biofilms (*cf.* Sect. 9.7.3), to

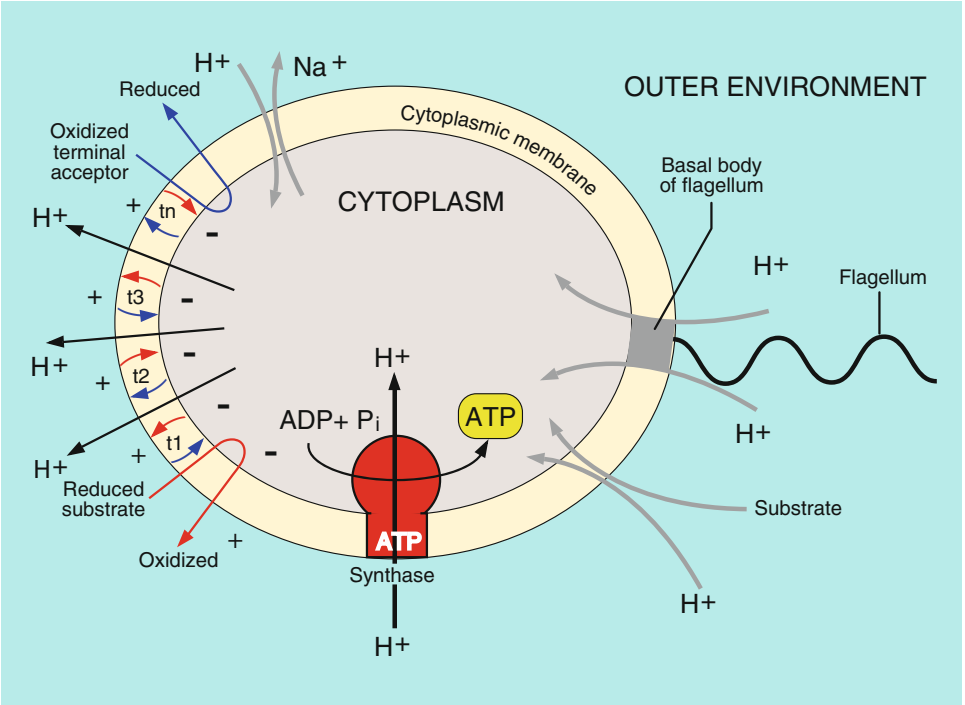

Fig. 3.4 Different modes of active transport and formation of a membrane proton gradient. t1 to tn: electron carriers (Drawing: M.-J. Bodiou)

adhere to surfaces, and to resist more or less effectively to environmental stresses. In some pathogenic bacteria, the capsule confers an additional pathogenicity, increasing the resistance to phagocytosis by the host organism.

Finally, some bacteria and archaea have a layer of proteins or glycoproteins on the outer surface of their walls, called S-layer that increases the protection of cells against environmental constraints.

3.1.1.2 Cytoplasm and Nucleoid

The cytoplasm is an aqueous solution of mineral salts and organic molecules necessary for the metabolic activity. It contains ribosomes, small particles of 15 nm in diameter, composed of proteins and ribosomal RNAs. Ribosomes are the site of the cellular protein synthesis and are composed of two subunits: the 50S subunit formed by proteins and 23S and 5S rRNAs and 30S subunit that consists of protein and 16S rRNA. The cytoplasm may contain inclusions of organic substances of reserves (glycogen, poly-β-hydroxyalkanoates, lipids) or inorganic compounds (polyphosphate, sulfur, iron) depending on the microbial type and metabolic activity. Other inclusions are more specific to some bacterial groups:

1. Cyanophycin granules, polypeptides constituting reserves of nitrogen in cyanobacteria.
2. Gas vacuoles for buoyancy of some prokaryotes.
3. Bacterial microstructures as carboxysomes, small particles containing ribulose 1,5-bisphosphate carboxylase necessary to the CO_2 fixation in some autotrophic prokaryotes.
4. Magnetosomes (*cf.* Sect. 14.5.2, Fig. 14.46) (containing magnetite). These structures provide magnetotactic bacteria the ability to move in a magnetic field.
5. Thylakoids, membrane structures forming flattened vesicles, location of the photosynthetic activity in cyanobacteria.
6. Chlorosomes, ovoid structures adjacent to the cytoplasmic membrane in phototrophic green bacteria, containing photosynthetic pigments (Fig. 3.27).

In some bacteria, protein filaments forming filamentous structures (cytoskeleton) in the cytoplasm under the cytoplasmic membrane may be involved in cell shape (Carballido-López 2006).

In a more or less central area of the cytoplasm, there is the nucleoid, consisting of a single double-stranded DNA molecule. It is the bacterial chromosome, usually single, circular, folded in on itself, and associated with proteins. Besides the chromosome, plasmids, small DNA molecules, can be present and transferred between cells in prokaryotes. Chromosome and extrachromosomal elements such as plasmids are part of the bacterial genome. Plasmids have few genes that are associated with specific properties of resistance (antibiotics, pollutants), virulence, or metabolism (biodegradation of xenobiotics). In bacteria belonging to the order *Planctomycetales*, an intracytoplasmic membrane divides the cytoplasm into two compartments, the peripheral paryphoplasm containing no ribosome and the central riboplasm containing ribosomes and the nucleoid (Fig. 14.32). In some of them, a

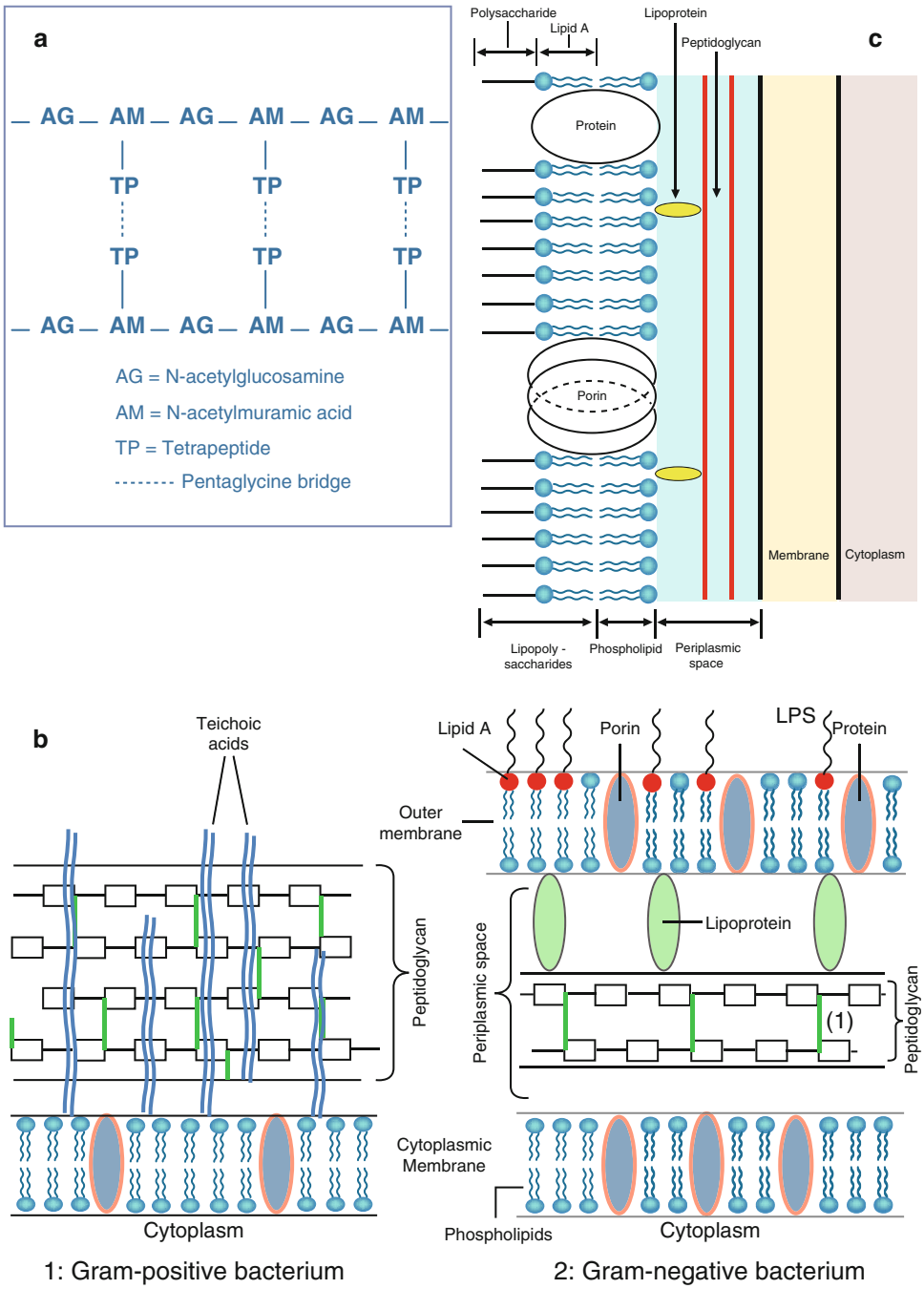

Fig. 3.5 The cell wall of bacteria. (**a**) Structure of peptidoglycan. (**b**) Cell wall structure of Gram-positive and Gram-negative bacteria. (**c**) Structure of the outer membrane of Gram-negative bacteria. (*1*) Pentaglycine bridges (Gram-positive bacteria) or direct bond between two amino acids of tetrapeptides (Gram-negative bacteria) (Drawing: M.-J. Bodiou)

nuclear envelope consisting of a double membrane surrounds the nucleoid inside the riboplasm, surprising discovery in prokaryotes, although this structure is not a true nucleus in the eukaryotic sense (Ward et al. 2006).

3.1.1.3 Spores and Cellular Appendages

Some bacteria are able to produce endospores of resistance when their environmental conditions become unfavorable. The endospores are resistant structures to extreme conditions (temperature, UV, pH, redox potential, drought, etc.). They are a means of survival and can stay alive for very long periods estimated at thousands of years. The endospore contains genetic material and a very dehydrated cytoplasm that are protected by a complex system consisting of several resistant envelopes (Fig. 3.6a). Other structures of resistance exist in prokaryotes such as **akinetes*** of C*yanobacteria*, cysts of *Azotobacter* and *Myxobacteria*, or exospores of *Actinobacteria*.

Flagella are filamentous structures 15–20 nm in diameter in the form of rigid spirals, which are composed of proteins, principally flagellin (Fig. 3.6b). They are inserted on a basal body formed of protein rings included in the envelopes of the

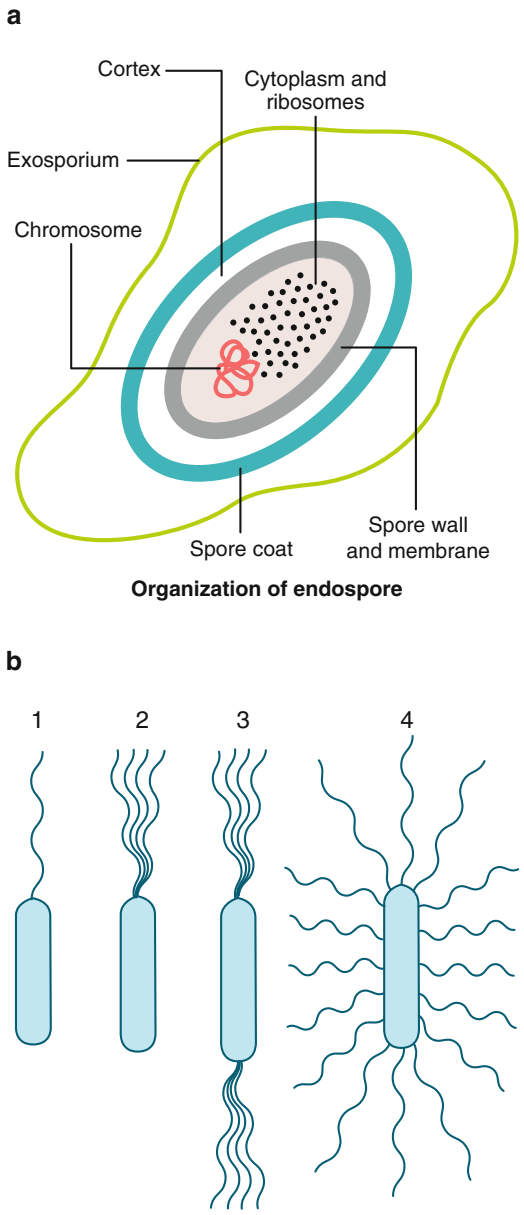

Fig. 3.6 Schematic representation of a bacterial endospore (**a**) and flagella and their location (**b**) (Drawing: M.-J. Bodiou)

cell. The activation of the basal body with membrane energy from the proton gradient allows rotation flagellum that drives the movement of the cell (Fig. 3.4). According to the bacteria, the implantation of flagella can be on one end (polar monotrichous or lophotrichous), at both ends (bipolar or amphitrichous), or on the whole bacterial cell (peritrichous). Spirochetes, spiral bacteria, do not possess a real flagellum but one axial filament resulting from the agglomeration of the flagella in the periplasm. Some bacteria move without flagellum, by sliding on their support ("gliding bacteria," *cf.* Sect. 9.7.2).

Some pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Erwinia* spp., etc.) possess secretion systems (e.g., type III or bacterial injectisome) in their envelopes, allowing them to inject cytoplasmic proteins in the cytosol of infected cells.

Intercellular nanotubes (diameter between 30 and 130 nm) have been described as forming conduits for exchanges of molecules between bacterial cells of the same species or different species growing on a solid surface (Dubey and Ben-Yehuda 2011).

Fimbriae or pili are present in bacteria. These are rigid filaments, thinner than flagella (3–7 nm in diameter), that play a role in cell adhesion to surfaces.

Finally, sex pili (10 nm in diameter) are produced by some bacteria to allow binding between bacteria and the formation of a cytoplasmic bridge used to transfer genes in conjugation between two bacterial cells. They provide the transfer of genetic material from one donor bacterium producing pilus to a receiver bacterium (*cf.* Chap. 12).

3.1.2 Eukaryotic Microorganisms (*cf.* Chaps. 5 and 7)

The cellular organization of eukaryotic microorganisms is much more complex than prokaryotic microorganisms. Eukaryotic microorganisms include photosynthetic and heterotrophic microorganisms that have important functional and morphological differences, but the basic cellular organization remains the same. Electronic micrograph of a cross section of eukaryote cell shows a central nucleus bounded by a nuclear membrane and surrounded by a cytoplasm containing structured membrane systems (Fig. 3.1b). The cytoplasm is surrounded by a membrane sometimes covered by a rigid wall in various taxa. The rigid wall is of chitinous, siliceous, or cellulosic nature. In some taxa, the cell is protected by a shell (theca) composed of protein, siliceous, or calcium substances. The phospholipid bilayer of the cytoplasmic membrane is asymmetrical. In the outer layer, glycolipids are inserted together with glycoproteins that serve as cellular receptors.

Sterols stabilize the membrane. In animal cells and many other taxa, the macromolecules and small particles penetrate through invaginations of the membrane (endocytosis) and are released into the cytoplasm as small vesicles or larger vacuoles (phagocytic vacuoles). The opposite (exocytosis) allows the release of cytoplasmic substances by fusion of intracellular vesicles with the cytoplasmic membrane. The nucleus is surrounded by a double phospholipid membrane (nuclear membrane) equipped with pores that allow exchanges with the cytoplasm. The interior of the nucleus

Table 3.2 Characteristic organelles of eukaryotic cells

Organelles	Description	Function
Endoplasmic reticulum (ER)	Flattened cavities (limited by smooth membranes or membranes covered with ribosomes) and vesicles	Synthesis of glycoproteins, lipids, sterols; transport of various substances
Ribosomes	80 S particles formed of two subunits of 40S and 60S	Synthesis of proteins, forming polysomes if arranged in chains fixed to mRNA
Golgi apparatus (GA)	Pile of flattened sacs formed from vesicles, derived from ER	Finishing synthesis (proteins), packaging, and secreting of various cellular substances (lysosomes)
Lysosomes	Set of vesicles from the ER and GA	Containing hydrolytic enzymes, digestion of substrates
Vacuoles	Origin: ER and GA; large size in plant cells	Contractile vacuoles, digestive vacuoles; sequestration and storage of substances; hydric balance of plant cells
Peroxisomes	Vesicles formed from the ER	Containing redox enzymes
Proteasome	Enzymatic complex	Digestion of endogenous proteins; regulation of enzyme activity
Mitochondria	Rod about 2–3 μm long, double membrane with folded inner membrane; containing DNA of prokaryotic type	Respiratory center of the cell, respiratory transporters, and enzymes included in the inner membrane
Chloroplasts	Various lamellar structures (thylakoids); containing DNA of prokaryotic type	Containing the photosynthetic pigments, photosynthesis
Centrioles	Formed by the association of microtubules	Formation of the centrosome; participate in the formation of undulipodia
Centrosome	Formed from centrioles	Intracellular movements
Undulipodia	Cytoplasmic expansions containing microtubules inserted on basal granules (kinetosomes)	Cellular movements

is occupied by chromatin, entanglement of double-stranded DNA molecules associated with proteins, histones. The chromosomes become visible at the time of cell division. In the middle of the chromatin, usually a single nucleolus is the location of the synthesis of ribosomal RNA.

The soluble portion of cytoplasm or cytosol contains a set of microfilaments and microtubules (cytoskeleton), involved in maintaining cell shape, intracellular movements, cell locomotion by forming pseudopods, and cellular exchanges (endocytosis and exocytosis). Many inclusions (droplets of lipids, polysaccharide reserve) and many organelles are located in the cytosol (Table 3.2). Some organelles are formed by double-layer phospholipid membrane, delineating compartments of various shapes (vesicles, tubules, lamellae, etc.). Among these organelles, some are limited by a single membrane (endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes), others by a double membrane (mitochondria and chloroplasts). There are also organelles composed of proteins: centriole, centrosome, ribosomes (RNAs and proteins), cilia, flagella (undulipodia), and proteasomes. Mitochondria and chloroplasts (plants), organelles of the same size as bacteria, have the particularity to contain DNA bacterial type (*cf.* Sects. 4.3.1 and 5.4.2). They multiply independently of the host cell. The amyloplasts of plants are specialized in synthesis and accumulation of starch. The presence of organelles and their importance in eukaryotic cells depends on the types of microorganisms and cellular activity.

Some eukaryotic microorganisms have a filamentous vegetative apparatus (Fungi). According to the taxa, these filaments are consisting of cells or a mass of multinucleated cytoplasm (syncytium). It may also be alternating between a single-cell generation and a filamentous generation (*cf.* Sect. 7.14.3).

3.2 Concept of Metabolism

The maintenance of cellular integrity, growth, and reproduction of living organisms requires the synthesis of cellular material, which depends on nutrients entering the interior of cells and subjected to a series of chemical changes. The sum of these changes is responsible for the production of energy (catabolism) and synthesis of biomolecules (anabolism); it is the metabolism of the cell (Fig. 3.7). Metabolic reactions are catalyzed by enzymes that, by combining with the biological molecules, decrease the activation energy of reactions and determine the reaction pathway to be used for the transformation of substances.

As sources of energy, the nutrient is oxidized, and the energy produced during the oxidation is transferred as energy-rich compounds, mostly in the form of ATP. These reactions of oxidation or degradation are the cellular catabolism:

1. In general, energy sources are organic compounds (**chemoorganotrophic microorganisms***).
   The oxidation of organic compounds can be partial or complete. When it is partial, the organic molecules of small molecular weight are produced, more oxidized than organic sources given as nutrients. The complete

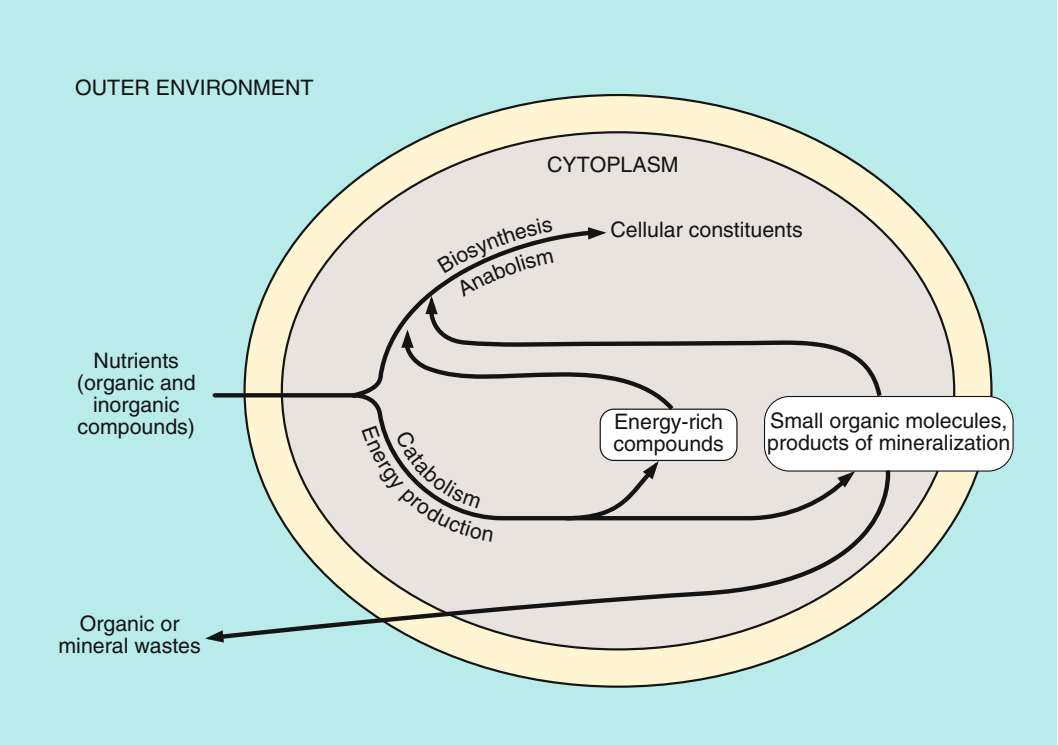

Fig. 3.7 Role of nutrients in cell metabolism (Drawing: M.-J. Bodiou)

oxidation results in formation of mineral compounds (CO_2, H_2O, NH_3, etc.). In this case, it is called a process of mineralization of organic sources.

Products of oxidation may remain inside the cell or be excreted out in the form of wastes. Thus, the chemoorganotrophic microorganisms are the decomposers of organic matter and ensure the progressive mineralization of organic matter, releasing mineral compounds in external environment (CO_2, NH_3, NO_3^-, PO_4^{3-}, SO_4^{2-}, HS^-).

2. The source of energy can be inorganic (**chemolithotrophic microorganisms***), consisting of reduced inorganic compounds such as dihydrogen, nitrogen or sulfur compounds, metals, etc.
3. The energy source can also be photonic (light) in the case of **photosynthetic*** or **phototrophic microorganisms*** which have pigments and photosynthetic systems able to react under the light action, thus converting light energy into chemical energy.

As sources of cellular constituents, the simple organic molecules produced in the cell or from the external environment are used as the basis of biosynthetic activities:

1. For most microorganisms, carbon organic compounds are required; these microorganisms are considered as **heterotrophs*.** They can use of low-weight organic molecules produced in energy reactions of degradation or taken from the environment.
2. Other microorganisms use CO_2 as sole carbon source to synthesize all their cellular components; these are **autotrophic microorganisms*** using a mineral source of energy (chemolithotrophic microorganisms) or light (phototrophic microorganisms).

In summary, several nutritional types are defined in microorganisms:

1. The energy source is:
   - *Chemical*: chemotrophic microorganisms using an organic source (**chemoorganotrophs***) or an inorganic source (**chemolithotrophs***)
   - *Photonic*: phototrophic microorganisms using an organic compound (**photoorganotrophs***) or a mineral compound (**photolithotrophs***) as a source of electrons
2. The carbon source is a compound:
   - *Organic*: heterotrophs
   - *Mineral* (CO_2): autotrophs

Cellular metabolism is formed by means of the redox reactions in catabolism for energy production (energy metabolism) and the reactions of biosynthesis necessary for anabolism (production of cellular components) (Fig. 3.8). To be functional and viable, a cell should maintain its internal environment in a reduced state in order to ensure cohesion and structure of macromolecules. For this, it must constantly produce energy and **reducing power*** during chemical or

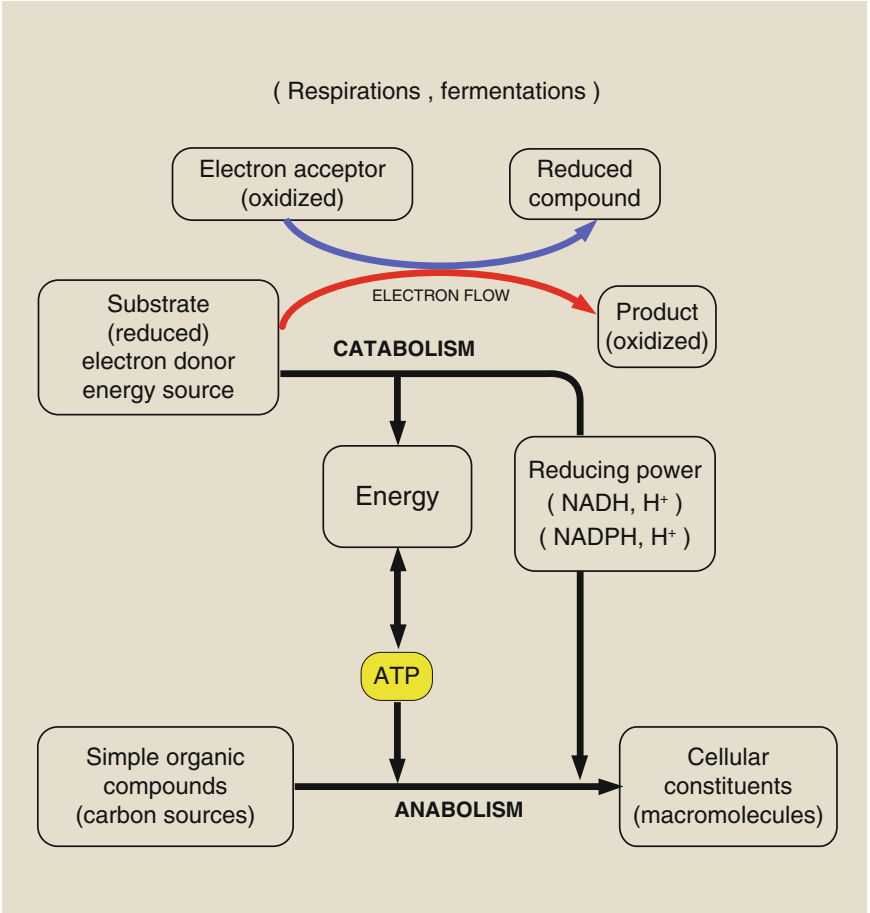

Fig. 3.8 General scheme of cellular metabolism (Drawing: M.-J. Bodiou)

photonic redox reactions. Too important processes of internal super-oxidation (oxidative stress, *cf*. Sect. 9.6.2) cause cell death.

For some microorganisms, all the needs of biosynthesis are covered by a single carbon source; these microorganisms are described as **prototrophs***. Others require, in addition to the main carbon source, organic compounds that cannot be synthesized such as amino acids, fatty acids, vitamins, etc. These compounds are considered essential for microorganisms and are called growth factors; microorganisms are then defined as **auxotrophs*** for a given essential compound.

At the level of a community of microorganisms in an ecosystem, the cellular catabolism corresponds to the role of decomposers or mineralizers, and anabolism corresponds to the role of producers of biomass for the food chain. Photosynthetic production of organic material by photosynthetic eukaryotes and *Cyanobacteria* (oxygenic photosynthesis) from CO_2 and H_2O is called **primary production*** because these organisms use an external source of energy to terrestrial ecosystems (light) and inorganic compounds which are not necessarily derived from the metabolism of other microorganisms. Primary producers synthesize organic matter from which all other biological activities depend. Similarly, anoxygenic phototrophic bacteria that use for their photosynthesis reduced mineral electron donors (sulfur compounds, reduced iron, dihydrogen) from geochemical activities are also considered as primary producers. In contrast, phototrophic or chemolithotrophic microorganisms using compounds from the degradation of organic matter cannot be defined as primary producers and are therefore referred to as **paraprimary producers***. This is the case of phototrophic chemoorganotrophic and chemolithotrophic bacteria using electron donors derived from microbial metabolism and chemolithotrophic autotrophic microorganisms that respire dioxygen resulting from photosynthesis and thus are depending on the actual primary producers. In natural environments, the degradation of organic matter from primary or paraprimary producers requires the intervention of a succession of mineralizing microorganisms exchanging produced and usable substrates (*cf*. Chap. 13).

Some molecules, especially xenobiotic products, can be degraded or transformed only in the presence of easily usable substrates as sources of carbon and energy. This phenomenon is described as the **cometabolism***.

When the growth of microorganisms is limited by another factor that the sources of carbon and energy, for example, the source of nitrogen, sulfur, or phosphorus, growth slows or stops, but the energy production rate is unaffected. This

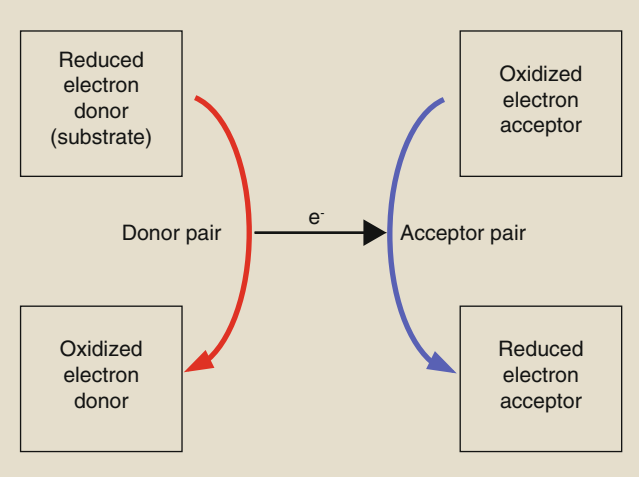

Fig. 3.9 Electron transfer between a donor and an acceptor in a redox reaction (Drawing: M.-J. Bodiou)

decoupling between growth and energy production is described as energy decoupling. The excess energy can be diverted to the production of glycogen and polyhydroxyalkanoates among microorganisms capable to accumulate these substances in their cells as carbon reserve.

3.3 Energy Metabolism

3.3.1 General Principles

In all cases (respiration, fermentation, photosynthesis), energy metabolism is based on redox processes between electron donor couple at low redox potential and electron acceptor couple at more high potential (Fig. 3.9).

During redox reactions that produce energy, the available energy for microorganisms appears mainly in the form of high-energy phosphate bonds, mainly in the form of adenosine triphosphate (ATP). ATP synthesis is an endergonic reaction that requires input of energy from the catabolism (potential phosphorylation: $\Delta Gp' = +44$ kJ.mol^{-1}). The released energy ($\Delta G^{\circ\prime}$) during the hydrolysis of ATP in ADP, which is –32 kJ.mol^{-1}, is used for biosynthesis and other cellular functions. ATP is not the only energy-rich molecule. Other molecules can be used such as phosphoenolpyruvate (PEP, $\Delta G^{\circ\prime} = -52$ kJ.mol^{-1}), acetyl phosphate ($\Delta G^{\circ\prime} = -45$ kJ.mol^{-1}), or acetyl-CoA ($\Delta G^{\circ\prime} = -36$ kJ.mol^{-1}).

In microorganisms, there are two types of mechanism for the synthesis of ATP, the **substrate-level phosphorylation*** and the phosphorylation during electron transfer by a carrier chain (**oxidative phosphorylation*** and **photophosphorylation***); these mechanisms involving the redox reactions of catabolism are associated often to transfer protons during dehydrogenation:

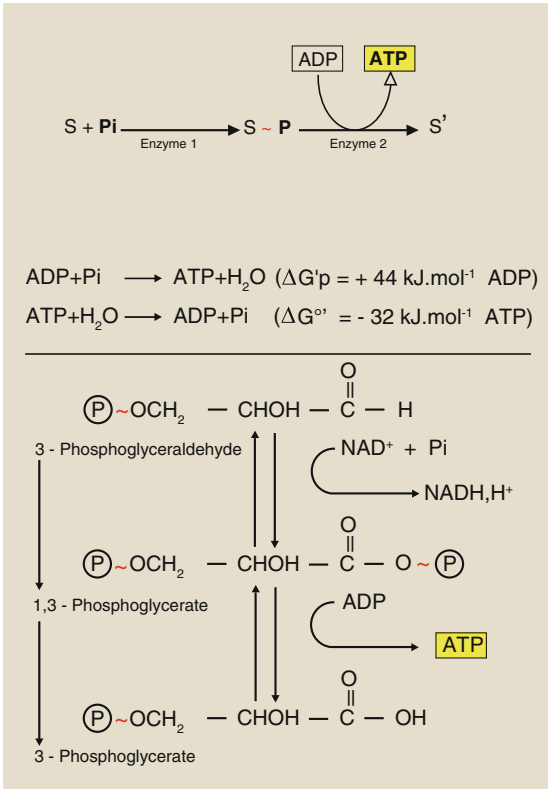

Fig. 3.10 Substrate-level phosphorylation. *Top*: Synthesis of ATP by phosphorylation of ADP and energy value of the ADP phosphorylation and dephosphorylation of ATP. *Bottom*: Example of substrate-level phosphorylation, phosphorylation glyceraldehyde in glycolysis. *Pi* inorganic phosphate, ~ energy-rich bond, *P* phosphate group (Drawing: M.-J. Bodiou)

1. Phosphorylation at the substrate level. The synthesis of ATP is directly coupled to the enzymatic oxidation of an organic substance. During the oxidation reaction, the organic substrate S is phosphorylated using an enzyme 1, and a phosphate ester with a high-energy bond (P ~ S) is produced. The high-energy phosphate is then transferred by means of an enzyme 2 to ADP to form ATP with an additional high-energy phosphate bond and releases a product S′ (Fig. 3.10). The enzymes involved in the phosphorylation at the substrate level are present in the cytoplasm and are soluble. The phosphorylation at the substrate level is the main mechanism of ATP production in fermentations.
2. Phosphorylation during electron transfer by a chain of membrane carriers. This is the mode of formation of ATP in the respiration (oxidative phosphorylation) and in the photosynthesis (photophosphorylation). During the oxidation—reduction reactions, electrons are transferred by a series of carriers from an initial electron donor with a low redox potential to a terminal electron acceptor at higher redox potential. Each carrier is characterized by a couple of redox (redox potential) between the oxidized and reduced forms (Fig. 3.11). During this transfer, electrons lose energy

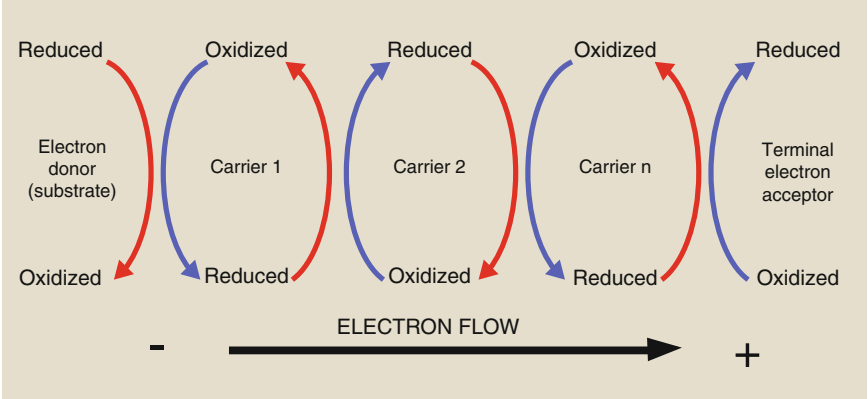

Fig. 3.11 General scheme of a chain of membrane electron carriers (Drawing: M.-J. Bodiou)

Table 3.3 Different types of respirations

Respiration in microorganisms	Electron donors	Final electron acceptors	Microorganisms
Aerobic chemoorganotrophs	Organic	Dioxygen	Prokaryotic and eukaryotic
Anaerobic chemoorganotrophs	Organic	Nitrate, sulfate, ferric iron, fumarate, etc.	Prokaryotic (sometimes eukaryotic)
Aerobic chemolithotrophs	Inorganic (ammonium, sulfide, ferrous iron, etc.)	Dioxygen	Prokaryotic
Anaerobic chemolithotrophs	Inorganic (ammonium, sulfide, hydrogen, etc.)	Nitrate, sulfate, etc.	Prokaryotic

which is recovered to synthesize ATP by phosphorylation of ADP. This synthesis is catalyzed by ATP synthases, **insoluble membrane enzymes***. During respiration, electron transfer between carriers takes place in the cytoplasmic membrane among prokaryotic microorganisms and in the internal mitochondrial membrane among eukaryotic microorganisms; the resulting ATP production is obtained by oxidative phosphorylation. For photosynthesis, electron transfer occurs in specialized membranes (thylakoids in cyanobacteria and chloroplasts in photosynthetic eukaryotes or photosynthetic cytoplasmic membrane in anoxygenic phototrophic bacteria), and ATP is produced by photophosphorylation. Almost all of enzymes associated with electron transport are included in the membrane and are insoluble. The transfer of electrons and protons generates a protonmotive force used as energy source or for the phosphorylation of ADP (Fig. 3.4).

3.3.2 Respirations in Microorganisms

The respiratory metabolism is more diverse in prokaryotic microorganisms than in eukaryotic microorganisms:

1. The energy source (electron donor) is necessarily organic in eukaryotes, while it may be organic or inorganic in prokaryotes (chemoorganotrophs or chemolithotrophs).
2. The terminal electron acceptor is usually dioxygen in eukaryotes, while in prokaryotes there are a variety of terminal electron acceptors in addition to dioxygen, such as nitrate, sulfate, iron, or manganese, thus defining a variety of anaerobic respirations.
3. The composition of respiratory chain (electron carriers) is variable in prokaryotes, while it is fairly constant in eukaryotes.

Thus, four types of respirations are known in microorganisms. Their characteristics are presented in Table 3.3.

Chemoorganotrophic microorganisms are generally heterotrophs, but some heterotrophs can use dihydrogen as donor of electrons. Chemolithotrophic microorganisms are mostly autotrophs, but some may use organic compounds as carbon sources. The microorganisms that use a mineral energy source and an organic carbon source are called **mixotrophic microorganisms***.

3.3.2.1 Aerobic Respiration in Chemoorganotrophic Microorganisms

Electrons or reducing equivalents (symbolized e^- or [H]) derived from the oxidation of a compound organic (electron donor or substrate) are transferred to dioxygen (terminal electron acceptor) by a chain of intermediate carriers (respiratory

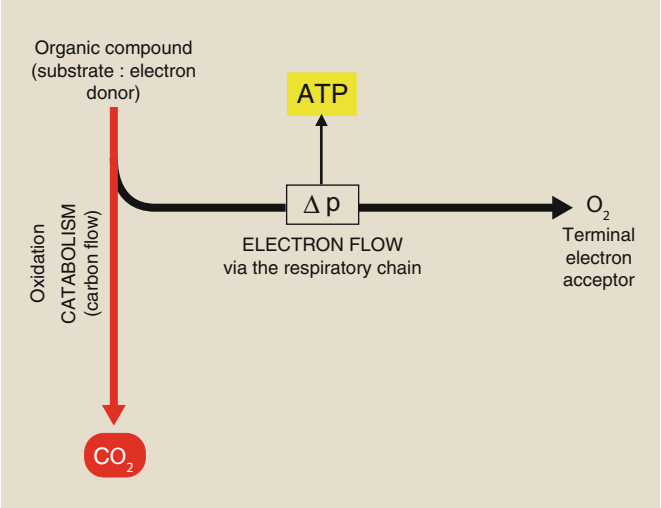

Fig. 3.12 Carbon flux and electron flow in aerobic respiration. Δp = Proton-motive force (Drawing: M.-J. Bodiou)

chain). During this transfer, energy is maintained particularly in the form of ATP produced by oxidative phosphorylation. The **reducing power*** or reducing equivalent sum that is produced during the oxidation of organic substrates is essentially reserved for energy production (Fig. 3.12). However, the reducing power operates in all cases where a reduction process is necessary: enzymatic reactions, biosynthesis, etc.

The oxidation reactions that take place in several steps are often reactions to dehydrogenation involving electrons and protons. The resulting reducing power is transferred to coenzymes (NAD^+, $NADP^+$, and FAD). There are many metabolic pathways of oxidation. For carbohydrates, the main pathway is glycolysis or the Embden–Meyerhof pathway associated with the tricarboxylic acid cycle (TAC), also called citric acid cycle, organic acid cycle to four carbon atoms (C4 cycle: succinic, fumaric, and malic acids), or the Krebs cycle.

Carbohydrate Oxidation

During glycolysis (Fig. 3.13a), glucose is oxidized to pyruvate in several enzymatic steps with concomitant formation of ATP by phosphorylation at the substrate level and reduced coenzymes:

$$\text{Glucose} + 2\text{ADP} + 2\ \text{Pi} + 2\ \text{NAD}^+ \rightarrow 2\ \text{pyruvate} + 2\ \text{ATP} + 2\text{NADH}, \text{H}^+$$

Pyruvate is then decarboxylated to acetyl-CoA and releases a molecule of CO_2 and reducing power ($NADH, H^+$):

$$2\ \text{pyruvate} + 2\ \text{CoA} + 2\ \text{NAD}^+ \rightarrow 2\ \text{acetyl} - \text{CoA} + 2\ \text{CO}_2 + 2\ \text{NADH}, \text{H}^+$$

The two molecules of acetyl-CoA are then oxidized via the Krebs cycle (Fig. 3.13b), by combining the acetyl group to a molecule of oxaloacetate to form a compound to six carbon atoms (citrate) that regenerates C4 compound (oxaloacetate) via a series of oxidation, decarboxylation, dehydration, and hydration reactions. During one cycle, the acetyl-CoA is oxidized to two molecules of CO_2 and allows the production of one molecule of ATP by phosphorylation at the substrate level, two molecules of NADH, H^+, one of NADP H, H^+, and one of $FADH_2$.

Thus, the total oxidation of glucose by glycolysis (Embden–Meyerhof pathway) and the Krebs cycle (Fig. 3.13c) produces six molecules of CO_2, four of ATP by phosphorylation at the substrate level, and 24 reducing equivalents [H] in the form of eight NADH, H^+, 2 NADP H, H^+, and two $FADH_2$. In prokaryotic microorganisms, glycolysis and the Krebs cycle take place in the cytoplasm, whereas in eukaryotes, glycolysis occurs in the cytoplasm and the Krebs cycle in the matrix of mitochondria.

Glucose can be degraded by other metabolic pathways such as the pentose phosphate methylglyoxal, phosphoketolase, and Entner–Doudoroff pathways.

Lipid Oxidation

Under the action of lipase, triglycerides are hydrolyzed to glycerol and fatty acids (Fig. 3.14a).

Glycerol enters into the Embden–Meyerhof pathway via the phosphoglycerate (Fig. 3.14b). The fatty acids are degraded by β-oxidation (Fig. 3.14c). During the β-oxidation, the fatty acid is esterified to acyl-CoA in the presence of coenzyme A. The acyl-CoA is then oxidized in position β in three steps (two dehydrogenations and one hydration). A new esterification in β releases a molecule of acetyl-CoA and a molecule of acyl-CoA that has lost two carbon atoms. A new round of β-oxidation begins. Finally, the fatty acid is cut into a succession of acetyl-CoA. These are metabolized through the glyoxylate cycle (Fig. 3.40).

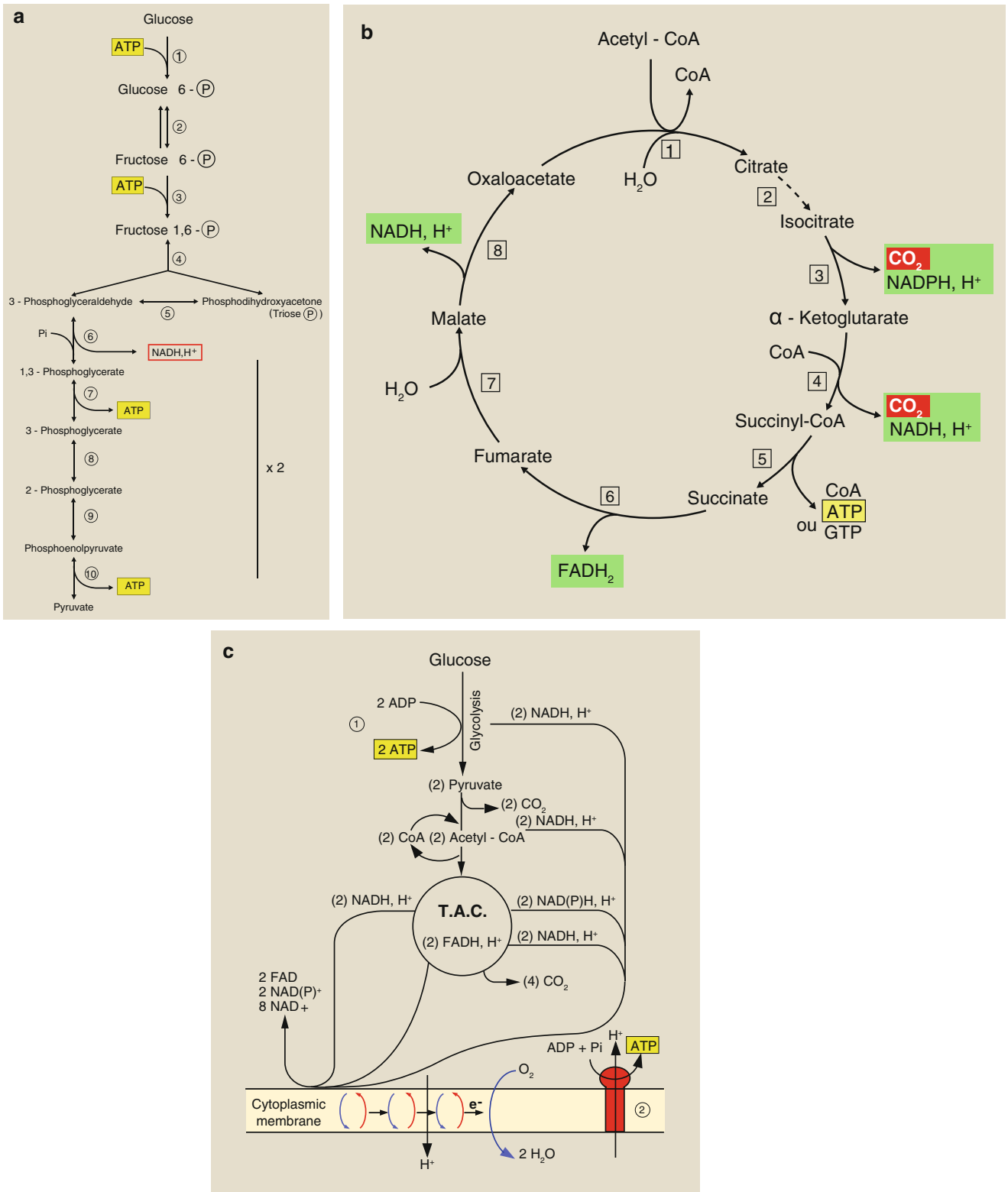

Fig. 3.13 Catabolism of glucose. (**a**) Glycolysis, a degradation pathway of glucose. *1* Glucokinase, *2* glucomutase, *3* phosphofructokinase, *4* aldolase, *5* isomerase, *6* 3-phosphoglyceraldehyde dehydrogenase, *7* 3-phosphoglycerate kinase, *8* phosphoglycerate mutase, *9* enolase, and *10* pyruvate kinase. *Pi* inorganic phosphate, *triose phosphates* 3-phosphoglyceraldehyde, and phosphodihydroxyacétone. (**b**) The Krebs cycle (tricarboxylic acid cycle, TAC). *1* Citrate synthase, *2* aconitate hydratase, *3* isocitrate dehydrogenase, *4* ketoglutarate dehydrogenase, *5* succinate thiokinase, *6* succinate dehydrogenase, *7* fumarase, and *8* malate dehydrogenase. (**c**) Scheme of glucose catabolism: glycolysis and Krebs cycle and electron transfer through the membrane respiratory chain. *1* Substrate-level phosphorylation, *2* oxidative phosphorylation via proton pores and ATP synthases (Drawing: M.-J. Bodiou)

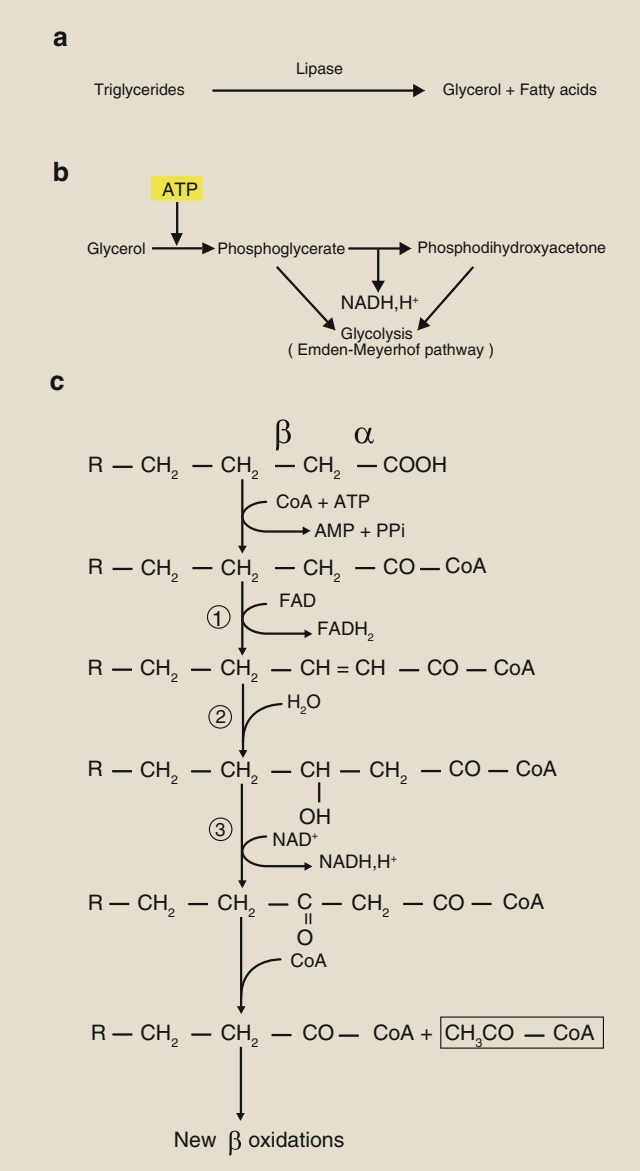

Fig. 3.14 Oxidation of lipids. (**a**) Enzymatic oxidation of triglycerides. (**b**) Incorporation of glycerol in glycolysis. (**c**) Fatty acid degradation by β-oxidation. *1* and *3* dehydrogenation reactions, *2* hydration reaction, *PPi* pyrophosphate (Drawing: M.-J. Bodiou)

With peer fatty acids, the last *β*-oxidation releases two acetyl-CoA, with odd fatty acids, one acetyl-CoA and one propionyl-CoA.

Protein Oxidation

Proteins are hydrolyzed by proteases to amino acids. Amino acids are then deaminated to organic acids that are oxidized via the Krebs cycle (Table 3.4).

Energy Synthesis

The synthesis of the energy is mainly produced at the level of the respiratory chain that catalyzes the oxidation of electron donors by transferring electrons to dioxygen (Fig. 3.15). The respiratory chain is located in the cytoplasmic membrane in prokaryotes and in the inner membrane of the mitochondria in eukaryotes. The respiratory chain consists of two types of carriers: electron and proton carriers (flavoproteins and quinones) and electron carriers only (protein Fe/S and cytochromes).

Table 3.4 Organic acids resulting from deamination of amino acids

Amino acids	Metabolic intermediates
Alanine, glycine, cysteine, serine, threonine	Pyruvate
Asparagine, aspartate	Oxaloacetate
Tyrosine, phenylalanine, aspartate	Fumarate
Isoleucine, methionine, threonine, valine	Succinate
Glutamate, glutamine, histidine, proline, arginine	*α*-Ketoglutarate
Isoleucine, leucine, tryptophan, lysine, phenylalanine, tyrosine	Acetyl-CoA

The respiratory chain receives electrons from reduced coenzymes (NADH, H^+, NADP H, H^+, and $FADH_2$) but also from some organic molecules (lactate, succinate, etc.). The reduced coenzymes are reoxidized by dehydrogenases, an enzymatic complex of the chain formed by the flavoproteins and Fe/S proteins. The electrons then pass through the quinones or ubiquinones, followed by a more or less complex pathway depending on the microorganisms, which comprises cytochromes, and finally by the cytochrome oxidase that transfers electrons to dioxygen which is reduced to H_2O. During this transport, protons are expelled ("proton translocation") outside of the cytoplasmic membrane in prokaryotes or in the intermembrane space of mitochondria in eukaryotes. The translocation of protons is due to the alternation of the two types of carriers in the respiratory chain and the role of proton pumps attributed to dehydrogenases and cytochromes (often cytochrome oxidases). The membranes are impermeable to protons; consequently, protons cannot return naturally in the cytoplasm, while electrons can return to the inner face of the membrane. It results in a charge distribution on both sides of the membrane causing a double gradient, pH and electric charge gradients, constituting the proton-motive force Δp (chemiosmotic theory of Mitchell) which is the main source of energy among aerobic chemoorganotrophic heterotrophic microorganisms. This energy will be used to produce ATP, the active transport of nutrients, cell movements, etc. The synthesis of ATP is due to return protons through pores associated with proton transmembrane enzyme complexes, the ATP synthases, which catalyze the phosphorylation of ADP to ATP.

If the organization of the mitochondrial respiratory chain is relatively constant, structure changes occur in aerobic prokaryotes mainly at the level of prokaryotic cytochromes and with environmental conditions (Fig. 3.16).

Differences in redox potential between electron donors and the final acceptor (dioxygen) define the quantity of free energy generated by electron transfer. For example, the calculated

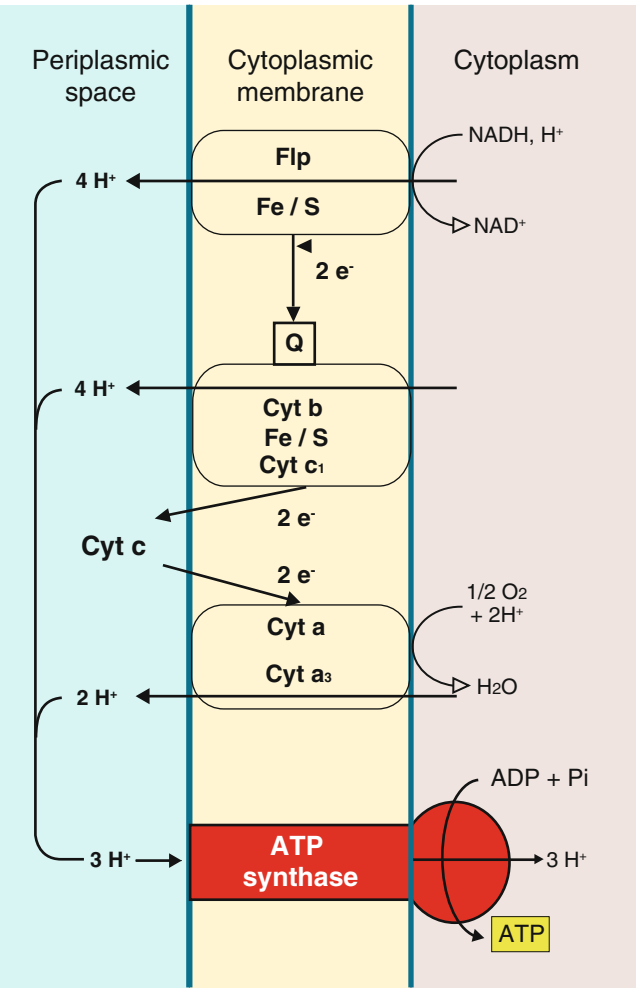

Fig. 3.15 Respiratory chain of aerobic chemoorganotrophic bacteria (*Paracoccus denitrificans*). *Flp* flavoprotein, *Fe/S* iron–sulfur protein, *Q* quinone, *Cyt* cytochrome, *complex [Flp-Fe/S]* NADH dehydrogenase, *complex [Q-Cyt b, Fe/S, Cyt c1]* catalyzes the recycling of electrons (quinone cycle), *Cyt c* cytochrome c oxidoreductase, *complex [Cyt a, Cyt a3]* cytochrome oxidase (Drawing: M.-J. Bodiou)

energy produced per mole of NADH is −220 kJ. The potential phosphorylation of ADP to ATP ($\Delta Gp'$) is 44 kJ.mol^{-1} ATP. Potentially, five moles of ATP could be synthesized in the transfer of two electrons to dioxygen from a mole of NADH, H^+. In fact according to the microorganisms, the actual energy yields are lower and, for example, can vary in general from 24 to 36 ATP per mole of glucose.

In general, the organic electron donor is also the source of carbon among chemoorganotrophic microorganisms that are also heterotrophs. In the case of *Escherichia coli*, about 50 % of the substrate is used for energy production, the rest acting as carbon source for biosynthesis; biosynthesis consumes the majority of produced cellular energy.

3.3.2.2 Aerobic Respiration in Chemolithotrophic Microorganisms

Chemolithotrophic microorganisms are all prokaryotes (*Bacteria* and *Archaea*) belonging to relatively small groups. They use oxidation of reduced inorganic compounds to produce energy by transferring electrons to the dioxygen via a membrane respiratory chain in the same way as that of chemoorganotrophic microorganisms (Figs. 3.17 and 3.18a–d).

Some bacteria are obligate chemolithotrophs, and others are facultative chemolithotrophs (can also use organic compounds). Many of chemolithotrophic bacteria use CO_2 as a carbon source and are chemolithoautotrophs, but some chemolithotrophs are heterotrophs for source of carbon and considered as chemolithoheterotrophs and are therefore mixotrophic microorganisms.

Main Electron Donors in Chemolithotrophic Microorganisms

The main reduced mineral compounds used as electron donors for chemolithotrophic prokaryotes are compounds of nitrogen and sulfur, iron, dihydrogen, and carbon monoxide.

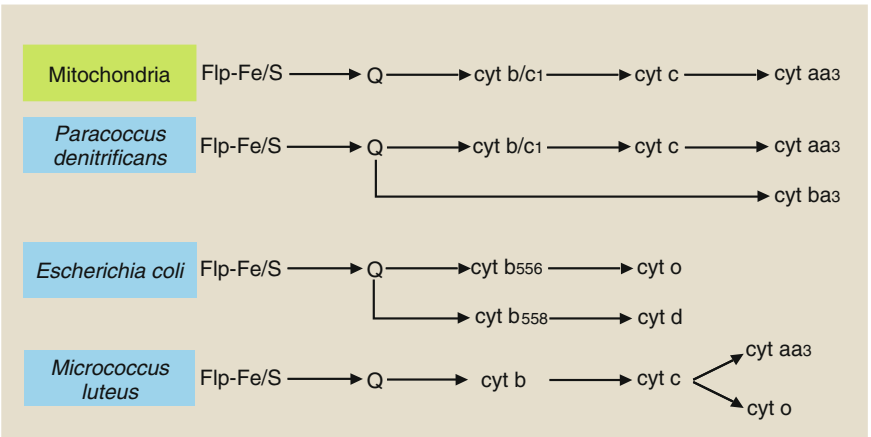

Fig. 3.16 Respiratory chains of different microorganisms. *Flp* flavoprotein, *Fe/S* iron–sulfur protein, *Q* quinone, *Cyt* cytochrome, Cyt b556, or cyt b558, wavelength in nm of the maximum absorption peak of light by cytochrome (Drawing: M.-J. Bodiou)

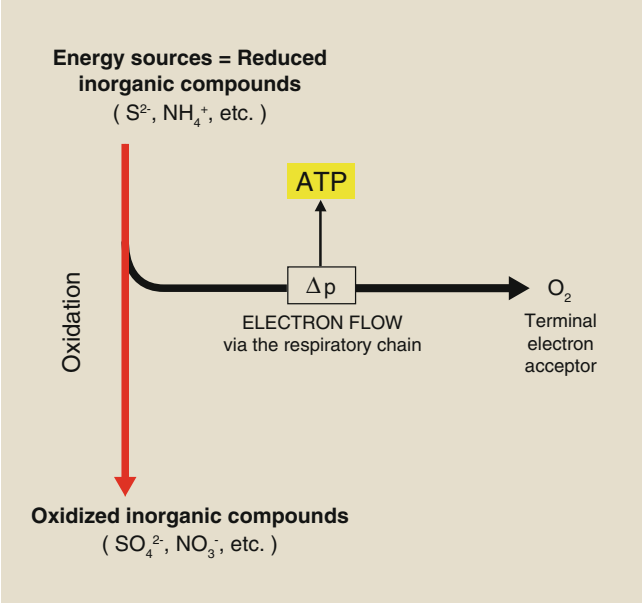

Fig. 3.17 General scheme of electron transfer in chemolithotrophic prokaryotes (Drawing: M.-J. Bodiou)

They can originate from aerobic or anaerobic degradation of organic matter or geochemical and biogeochemical processes (*cf.* Chap. 14).

The reduced nitrogen compounds are used by nitrifying bacteria that form a heterogeneous group including various genera and species all highly specialized in nitrification. Two large groups can be distinguished based on the reduced nitrogen compounds used by nitrifying bacteria: the group of ammonia-oxidizing bacteria that oxidize ammonia to nitrite (*Nitrosomonas*, *Nitrosococcus*, etc.) and the group of nitrite-oxidizing bacteria that oxidize nitrite to nitrate (*Nitrobacter*, *Nitrospira*, etc.). Most of these microorganisms are chemolithoautotrophs with the exception of some mixotrophs such as *Nitrobacter* that can use acetate as carbon source. In the absence of dioxygen or in dioxygen-limiting conditions, *Nitrosomonas europaea* can express a denitrifying activity. It was shown that ammonia-oxidizing archaea were abundant in natural environments.

Reduced sulfur compounds, mainly sulfide (S^{2-}or HS^-or H_2S), elemental sulfur (S°), thiosulfate ($S_2O_3^{2-}$),

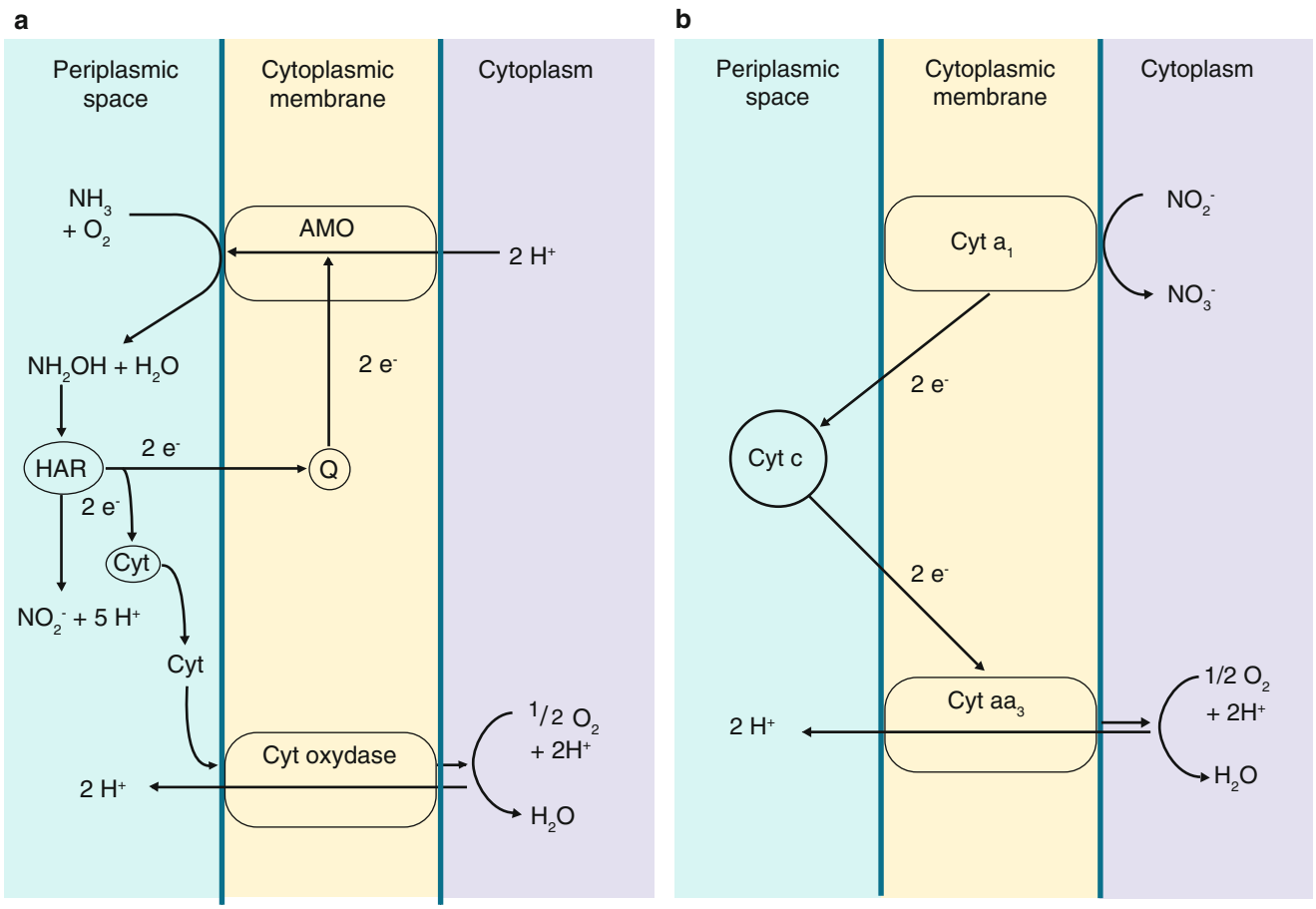

Fig. 3.18 Schemes of the respiratory chains of chemolithotrophic bacteria: ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, sulfur-oxidizing bacteria, and iron-oxidizing bacteria. (**a**) Respiratory chain of *Nitrosomonas* (Modified and redrawn from Hooper et al. 1997). *AMO* ammonia monooxygenase, *NH_2OH* hydroxylamine, and *HAR* hydroxylamine oxidoreductase. (Note: Energy is necessary for the first stage, which is provided by the return of two electrons to AMO). (**b**) Respiratory chain of *Nitrobacter*.

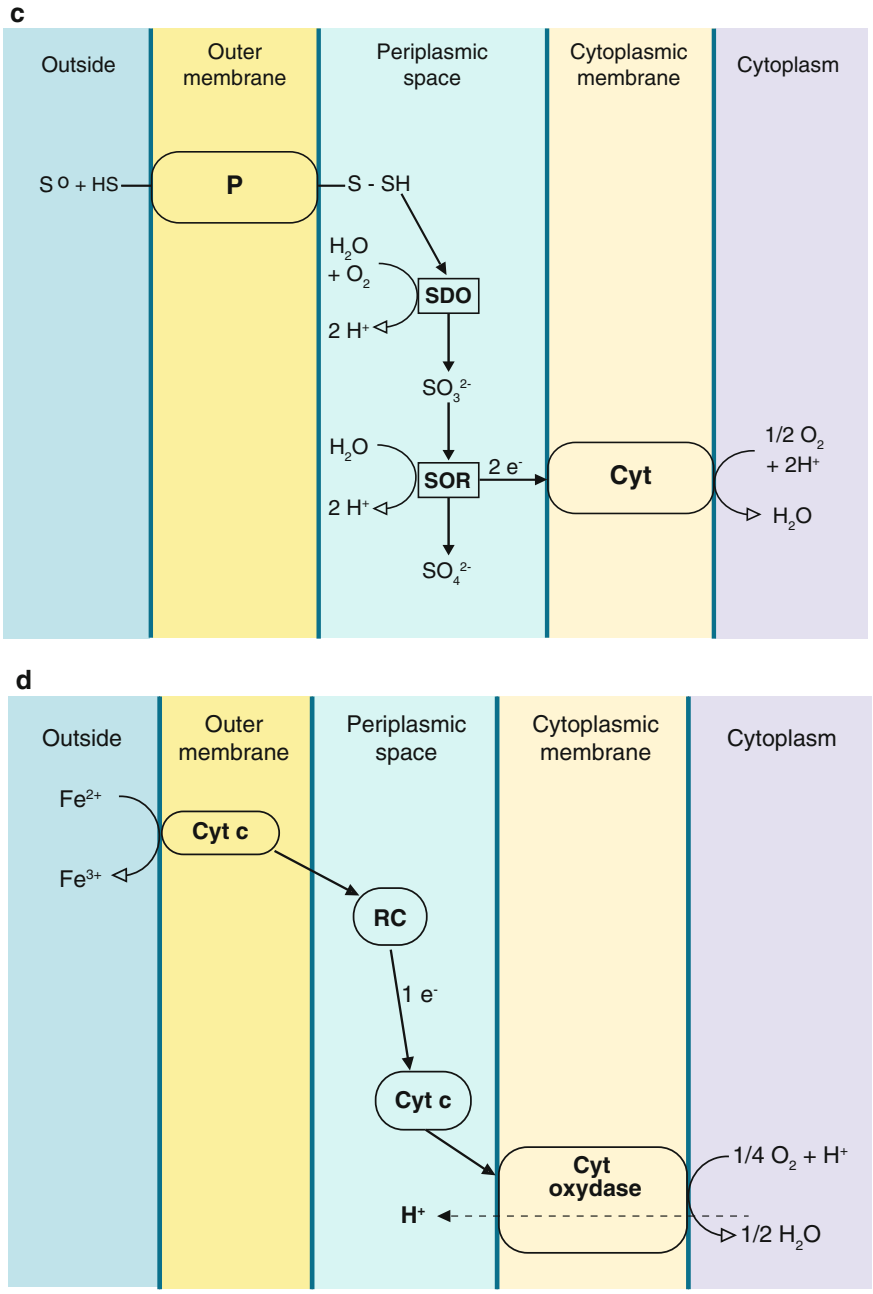

Fig. 3.18 (continued) The values of the redox potentials do not allow the transfer of electrons between the cytochrome a1 ($Eo' = + 0.35$ V) and c ($Eo' = + 0.27$ V). However, high oxidase activity of cytochrome aa3 maintains cytochrome c in a highly oxidized state that renders possible the transfer. (**c**) Respiratory chain of *Acidithiobacillus* (Modified and redrawn from Rohwerder and Sand 2003). The sulfur atom (S °) is mobilized in the form of persulfide by reaction with a thiol group of a protein (P) of the periplasmic membrane. Only two of six electrons involved are transferred by the respiratory chain to dioxygen. *SDO* sulfur dioxygenase, *SOR* sulfite oxidoreductase, and *Cyt* cytochrome(s). (**d**) Respiratory chain of *Acidithiobacillus ferrooxidans* (Modified and redrawn from Valdés et al. 2008). Cytochrome oxidase: cytochrome oxidase, *Cyt c* cytochrome c, and *RC* rusticyanin (copper protein) (Drawing: M.-J. Bodiou)

tetrathionate ($S_4O_6^{2-}$), and sulfite (SO_3^{2-}), are used by aerobic sulfur-oxidizing bacteria (or colorless sulfur-oxidizing bacteria) described as colorless compared to the phototrophic purple and green sulfur-oxidizing bacteria. Among aerobic sulfur-oxidizing bacteria, some are obligate chemolithotrophs, others are facultative chemolithotrophs, others are autotrophs, others are heterotrophs, and even some are obligate chemoorganotrophic heterotrophs (Table 3.5). Two major

Table 3.5 Different metabolisms of colorless sulfur bacteria and archaea

Trophic types	Energy source		Carbon sources		Microorganisms[a]	Growth pH	
	Inorganic	Organic	Inorganic	Organic		Neutral	Acidic
Obligatory autotrophic chemolithotrophs	X		X		*Thiobacillus*[b], *Acidithiobacillus*[b], *Acidianus*[c], *Sulfolobus*[c], *Hydrogenobacter*[b], *Thiomicrospira*[b]	X	X
Facultative autotrophic chemolithotrophs	X	X	X	X	*Thiobacillus*[b], *Sulfolobus*[c], *Acidianus*[c], *Thiosphaera*[b] *Paracoccus*[b], *Metallosphaera*[c], *Beggiatoa*[b]	X	X
Chemolithoheterotrophs	X	X		X	*Thiobacillus*[b], *Beggiatoa*[b]	X	
Chemoorganoheterotrophs		X		X	*Beggiatoa*[b], *Macromonas*[b], *Thiobacterium*[b], *Thiothrix*[b]	X	

[a]At least one species of the mentioned genus is concerned with the physiological characteristics
[b]*Bacteria*
[c]*Achaea*

groups are distinguished morphologically: the group of unicellular bacteria (*Thiobacillus*, *Thiospira*, etc.) and the group of filamentous bacteria (*Beggiatoa*, *Thiothrix*, etc.). Most bacteria are neutrophiles and some are extreme acidophiles (*Acidithiobacillus*). *Sulfolobus* is one of thermophilic and acidophilic sulfur-oxidizing archaea. During the oxidation of sulfur compounds, sulfur-oxidizing bacteria form globules of sulfur accumulated outside (*Thiobacillus*, *Acidithiobacillus*) or inside the cells (*Beggiatoa*, *Sulfolobus*, etc.). *Acidithiobacillus ferrooxidans* can also use iron (Fe^{2+}) as electron donor in extreme acidophilic conditions. The accumulated sulfur by aerobic sulfur-oxidizing bacteria can form large deposits.

The reduced iron or ferrous iron (Fe^{2+}) is abundant in nature, but only few microorganisms are capable to use mainly because of its low solubility in water and because of its rapid oxidation by the dioxygen at neutral pH. It is used by aerobic bacteria as an electron donor and is oxidized to ferric iron (Fe^{3+}) via a respiratory chain which transfers electrons to dioxygen (Fig. 3.18d). At neutral pH, few iron bacteria are able to use ferrous iron (*Gallionella*, *Mariprofundus*) and are obligate chemolithotrophic autotrophs. At acidic pH, ferrous iron is more soluble and chemically stable. It is a source of usable energy for acidophilic iron-oxidizing bacteria such as *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* which are obligate chemolithoautotrophs, or *Acidimicrobium* which is facultative chemolithoautotroph. These bacteria live at a pH between pH 1 and pH 3. The thermoacidophilic archaeal *Sulfolobus* is also able to use ferrous iron.

Aerobic iron-oxidizing bacteria and aerobic sulfur-oxidizing bacteria are restricted to aerobic interfaces between the **oxic*** and **anoxic zones*** because the used reduced compounds are generally spontaneously oxidized in the presence of dioxygen and can only be maintained in the reduced state in anoxic areas.

Biotic or abiotic (hydrothermal) dihydrogen is used as a donor of electrons by a large number of aerobic or microaerophilic microorganisms which have a membrane hydrogenase acting in oxidation of dihydrogen and release of protons. The protons excreted contribute to the formation of a proton gradient, and electrons are transferred to dioxygen via a membrane respiratory chain. The hydrogenase is sensitive to dioxygen; also many bacteria are **microaerophiles*** and live at only low dioxygen tension (1–5 %). These microorganisms are facultative chemolithoautotrophic bacteria belonging to various genera such as *Pseudomonas*, *Alcaligenes*, *Nocardia*, *Gordonia*, *Hydrogenophaga*, etc. Some of them are obligate chemolithotrophic autotrophs (*Hydrogenovibrio*, *Hydrogenothermus*, *Aquifex*):

$$H_2 \rightarrow 2\,H^+ + 2\,e^-$$

Carbon monoxide (CO) present in large amounts in habitats rich in organic matter and low in O_2 (e.g., rice paddies) is metabolized by a small number of microorganisms which also generally use dihydrogen as donor electrons and possess a CO dehydrogenase transferring the electrons from the oxidation of CO to CO_2 to a membrane respiratory chain. These are facultative chemolithotrophic bacteria such as *Oligotropha carboxidovorans*, *Pseudomonas carboxydohydrogena*, *Mycobacterium* spp., or some *Bacillus* (*Bacillus schlegelii*).

Other compounds may serve as electron donors for chemolithotrophic microorganisms in order to produce energy, such as the reduced compounds of copper, manganese, antimony, selenium, or arsenic. These compounds are often toxic, and bacteria that use them must be able to resist to their toxicity.

Energy Production in Chemolithotrophic Microorganisms

The electrons from the oxidation of reduced mineral compounds are transferred to dioxygen via a respiratory chain located in the cytoplasmic membrane. The redox potential of these mineral compounds is generally too high; the respiratory chain described previously in chemoorganotrophic microorganisms is not used in its entirety but only

Table 3.6 Redox potentials of redox couples and $\Delta G^{\circ\prime}$ of the oxidation reactions of various energy sources utilized by chemolithotrophic microorganisms

Redox pair	Eo′ in volts	$\Delta G^{\circ\prime}$ in kJ/2 electrons
CO_2/CO	−0.52	−258.6
H^+/H_2	−0.42	−239.3
SO_4^{2-}/HS^-	−0.22	−200.7
NO_2^-/NH_4^+	+0.34	−92.6
NO_3^-/NO_2^-	+0.43	−75.3
Fe^{3+}/Fe^{2+}	+0.77	−9.6[a]
O_2/H_2O	+0.82	
$NAD^+/NADH$	−0.32	−219.9

[a]$\Delta G^{\circ\prime}$ to pH 7; ΔG° is −65.8 kJ/2e$^-$ at physiological pH of *Acidithiobacillus ferrooxidans* (pH 2)

in its terminal part (cytochromes, Fig. 3.18a–d). Thus, the potential difference between the donor couple (reduced mineral compound) and acceptor couple (O_2/H_2O) (Table 3.6) is relatively low, so the amount of energy will always be low except for paths using dihydrogen or CO as electron donors. As a result, the chemolithotrophic bacteria oxidize a large amount of reduced compounds (electron donors) for a relatively low energy and therefore biomass. In the case of sulfur-oxidizing bacteria and nitrifying bacteria, this great oxidation activity can lead a significant production of acid compounds (H_2SO_4, HNO_3) which, in environments poorly buffered, are responsible for the phenomena of corrosion.

The translocation of protons resulting from activity of the respiratory chain leads to a proton-motive force that can be used as energy or allow the synthesis of ATP by oxidative phosphorylation. In most chemolithotrophic bacteria, ATP production is exclusively obtained by oxidative phosphorylation, with the exception of sulfur-oxidizing bacteria in which a small amount of ATP is produced by substrate-level phosphorylation in the oxidation of sulfite to sulfate.

In chemolithotrophic autotrophic microorganisms, the reduction of CO_2 into organic compounds used for biosynthesis requires reducing power in the form of large amounts of reduced coenzymes (NADH, H^+ or NADPH, H^+). The electrons required for reduction of these coenzymes are derived from the oxidation of electron donor (reduced inorganic compound). However, apart from the case of dihydrogen and CO, the redox potentials of redox couples of electron donors (energy sources) are higher than that of redox couple of $NAD^+/NADH$, H^+, so electrons cannot be transferred spontaneously to NAD^+. The result is a transfer by an **electron reverse flow*** through a portion of the respiratory chain that consumes energy (Fig. 3.19). The role of energy is to place the electrons in an energy level sufficient to reduce the coenzymes. Thus, the small amount of energy produced by respiration in chemolithotrophic bacteria is largely used to produce reducing power (via the intermediary of the reverse electron flow) required for biosynthesis.

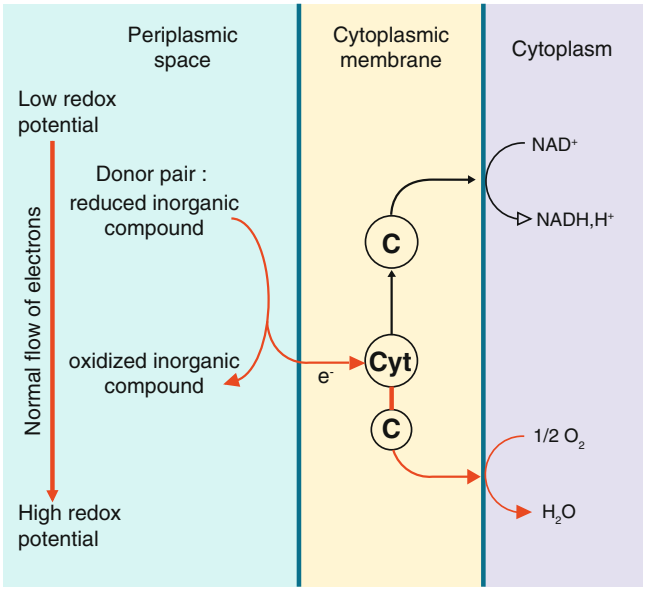

Fig. 3.19 General scheme of reverse electron flow in chemolithotrophic bacteria. C = electron carriers. The reverse flow corresponds to the electron transport from cytochromes (cyt) to NAD^+ via membrane carriers (C) (Drawing: M.-J. Bodiou)

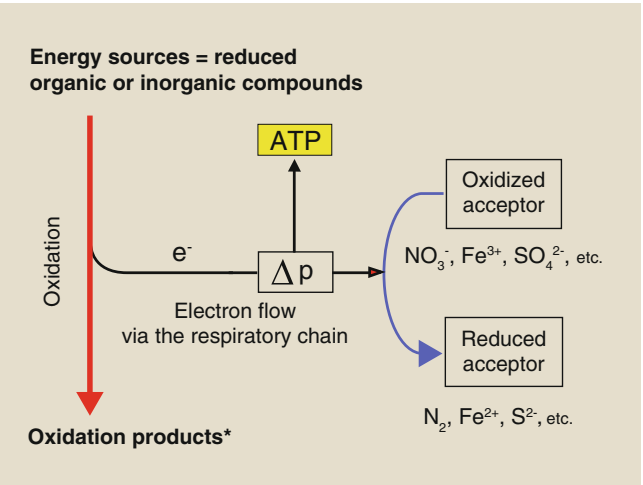

Fig. 3.20 General scheme of anaerobic respirations. Oxidation of organic energy sources can be complete (CO_2) or partial (Drawing: M.-J. Bodiou)

3.3.2.3 Anaerobic Respirations

In anoxic environments and in the absence of light, microorganisms can use two systems that produce energy, fermentations and anaerobic respirations. During anaerobic respirations, mechanisms of energy conservation are very similar to aerobic respiration (Fig. 3.20). However, during these respirations, the electrons from the oxidation of electron donor (substrate) are not transferred through the respiratory chain to dioxygen but to other oxidized compounds which act as terminal electron acceptor (Table 3.7). These inorganic (nitrate, sulfate, ferric iron, CO_2, chlorate, etc.) or sometimes organic

Table 3.7 Anaerobic respirations in prokaryotes and eukaryotes

Main anaerobic respirations[a] of prokaryotes[b]	Terminal electron acceptors
Iron respiration (b)	$Fe^{3+} + e^- \rightarrow Fe^{2+}$
Nitrate respiration (e.g., denitrification) (b, a)	$NO_3^- + 5\ e^- \rightarrow N_2$
Sulfate respiration (sulfate reducing) (b, a)	$SO_4^{2-} + 8\ e^- \rightarrow S^{2-}$
Sulfur respiration (sulfur reducing) (b, a)	$S^\circ + 2\ e^- \rightarrow S^{2-}$
CO_2 respiration (acetogenesis) (b)	$CO_2 + 2\ e^- \rightarrow$ acetate
CO_2 respiration (methanogenesis) (a)	$CO_2 + 4\ e^- \rightarrow CH_4$
Fumarate respiration (b)	Fumarate + 2 e^- → succinate
Anaerobic respirations of eukaryotes	Main microorganisms
Fumarate respiration	*Euglena*, *Leishmania*
Nitrate respiration	*Fusarium*, *Cylindrocarpon* (Fungi)

[a]*Bacteria* (b), *Archaea* (a)
[b]Other electron acceptors for anaerobic respirations in prokaryotes: manganese oxide (MnO_2), arsenate (AsO_4^{3-}), selenate (SeO_4^{2-}), chromate (CrO_4^{2-}), vanadium oxide (V_2O_5), dimethyl sulfoxide, organic chloride compounds, trimethylamine oxide, and chlorate

terminal acceptors (fumarate, dimethyl sulfoxide, chloride organic compounds) are reduced. The reduction of the terminal acceptor is catalyzed by different reductases associated with a respiratory chain. When the electron donor is an organic compound, its oxidation may be complete, releasing CO_2, or partial. The product of the reduction of the terminal acceptor is excreted in the external environment and is not used to be assimilated in the biosynthesis, and thus anaerobic respirations are **dissimilatory reductions*** of terminal electron acceptors. However, all dissimilatory reductions are not anaerobic respirations. Some bacteria eliminate an excess of reducing power by transferring electrons to mineral acceptors.

According to the redox potentials of terminal electron acceptor couple (Table 3.6), the energy generated by electron transfer during anaerobic respiration will be different. Indeed, the energy production will be all larger than the terminal electron acceptor has a redox couple with a high potential. This is the case for nitrate and iron respirations, whose redox couples are close to the O_2/H_2O couple. Thus, for most conventional anaerobic respiration, a series of electron acceptors in order of decreasing energy production is as follows:

$$O_2 > Fe^{3+} > NO_3^- > Mn_4^+ > SO_4^{2-} > CO_2$$

In an anoxic environment, according to the available electron acceptors, it is always the anaerobic respiration which produces the most available energy that dominates in the anaerobic microbial community. This rule is always verified if the terminal electron acceptor is present under non-limiting conditions. Anaerobic respirations are present in all three domains of life, but they are especially important and common in prokaryotic domains (*Bacteria* and *Archaea*).

Table 3.8 Some genera of microorganisms containing denitrifiers

Eukaryotes	Examples of microorganisms
	Globobulimina[a], *Cylindrocarpon*[b], *Fusarium*[b]
Prokaryotes	Examples of microorganisms
Archaea	*Ferroglobus*, *Haloarcula*, *Halobacterium*, *Haloferax*, *Pyrococcus*
Bacteria	*Nitrobacter*, *Paracoccus*, *Pseudomonas*, *Rhizobium*, *Rhodopseudomonas*, *Thiobacillus*, *Alcaligenes*, *Neisseria*, *Nitrosomonas*, *Zoogloea*, *Acinetobacter*, *Marinobacter*, *Shewanella*, *Wolinella*
	Bacillus, *Frankia*, *Nocardia*
	"*Candidatus* Kuenenia stuttgartiensis" (anammox)

[a]Foraminifera
[b]Fungi

Dissimilatory Nitrate Reduction

The use of nitrate (the most oxidized nitrogen, oxidation state + V) as terminal electron acceptor is widespread in prokaryotes (Table 3.8). The first reduction step leading to NO_2^- (+III) produces energy via the respiratory chain linked to proton translocation (Fig. 3.21). Subsequently, there are two possible futures for NO_2^-. It can accumulate in the environment, but it is often reduced to NH_3 (−III) especially among *Enterobacteriaceae*, *Staphylococcus*, and *Fusarium* (ammonia dissimilative reduction). NO_2^- may also be reduced to N_2 (oxidation state 0) in several steps via the respiratory chain. This is the denitrification (reduction of NO_3^- to N_2) in which each step is producing energy and forms gaseous compounds of nitrogen that can be released into the atmosphere (nitrogen loss) (*cf.* Sects. 14.3.3 and 14.3.5).

The different reduction steps are catalyzed by reductases: nitrate reductase Nar (3.1), NADPH-dependent nitrite reductase (3.2), nitrite reductase (3.3), nitric oxide reductase (3.4), and nitrous oxide reductase (3.5):

$$NO_3^- + 2\ e^- + 2\ H^+ \rightarrow NO_2^- + H_2O \qquad (3.1)$$

$$NO_2^- + 3\ NADPH, H^+ + H^+ \rightarrow NH_3 + 2H_2O + 3\ NADP^+ \qquad (3.2)$$

$$NO_2^- + e^- + 2\ H^+ \rightarrow NO + H_2O \qquad (3.3)$$

$$NO + e^- + H^+ \rightarrow ½\ N_2O + ½\ H_2O \qquad (3.4)$$

$$½\ N_2O + e^- + H^+ \rightarrow ½\ N_2 + ½\ H_2O \qquad (3.5)$$

The electrons necessary for reducing the nitrogen compounds in reactions (3.1), (3.3), (3.4), and (3.5) are produced during the oxidation of the substrate and transferred through the respiratory chain (Fig. 3.21). The amount of energy obtained by the nitrate respiration is very high,

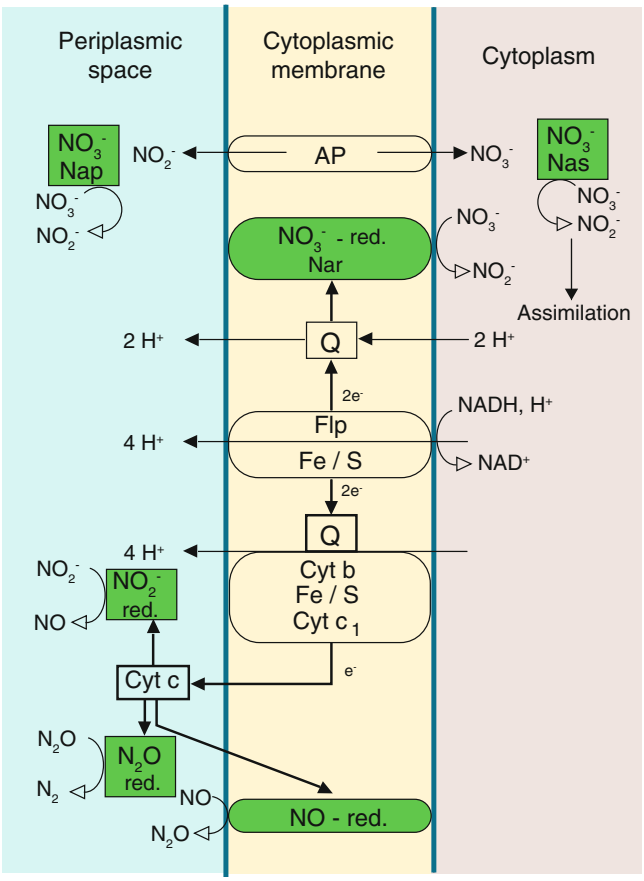

Fig. 3.21 Scheme of the respiratory chain of *Paracoccus denitrificans*, chemoorganotrophic denitrifying bacterium. *NO_3^- red Nar* dissimilatory nitrate reductase, *NO_3^- Nap* periplasmic nitrate reductase (aerobic denitrification), *NO_3^- Nas* assimilatory nitrate reductase, *NO_2^- red* nitrite reductase, *NO red* nitric oxide reductase, *N_2O red* nitrous oxide reductase, *AP* antiport transport system NO_3^-–NO_2^- (Drawing: M.-J. Bodiou)

close to that obtained by aerobic respiration. For example, with glucose as substrate,

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,H_2O + 6\,CO_2 \qquad \Delta G^{\circ\prime} = -2,868\ \text{kJ}$$
$$C_6H_{12}O_6 + 12\,NO_3^- \rightarrow 12\,NO_2^- + 6\,CO_2 + 6\,H_2O \qquad \Delta G^{\circ\prime} = -1,764\ \text{kJ}$$
$$C_6H_{12}O_6 + 4\,NO_3^- \rightarrow 2\,N_2 + 6\,CO_2 + 6\,H_2O \qquad \Delta G^{\circ\prime} = -2,425\ \text{kJ}$$

Prokaryotes that perform nitrate respiration are facultative anaerobic microorganisms, able to use the dioxygen in oxic conditions and nitrate under anoxic conditions. Enzymes that reduce nitrogen compounds are sensitive to dioxygen, and thus nitrate respiration will begin only when dioxygen is in very low concentration in the cell environment. Denitrifying enzymes have a sensitivity to dioxygen that is increasing from nitrate reductase which is tolerant to dioxygen, to nitrous oxide reductase, which is the most sensitive; so, when dioxygen is in low concentration, nitrate reduction can start but not the reduction of nitrous oxide whose reductase is inhibited at low-concentration dioxygen, thereby releasing nitrous oxide in the atmosphere (*cf.* Sect. 14.3.5, Fig. 14.31). In most bacteria that reduce nitrate, these different enzymes are inducible under anaerobic conditions and in the presence of nitrogen compounds, with the exception of some bacteria where they are constitutive (*Thiomicrospira denitrificans*). Like other carriers of the respiratory chain, the reductases are included into the cytoplasmic membrane with the exception of nitrite reductase and N_2O reductase which are periplasmic. In some bacteria, there is a constitutive periplasmic nitrate reductase (nitrate reductase Nap) that allows for a real aerobic denitrification. Prokaryotes that perform nitrate respiration are predominantly chemoorganotrophic heterotrophic microorganisms. Some are obligate or facultative chemolithotrophs. Among the denitrifying microorganisms, some do not have complete enzymatic equipment and cannot perform the full steps of denitrification.

Ettwig et al. (2010) described an "intra-aerobic" pathway for the reduction of nitrite and the oxidation of methane. In the bacterium "*Candidatus* Methylomirabilis oxyfera," the final reduction of nitrite to dinitrogen involves the conversion of two molecules of nitric oxide in dinitrogen and dioxygen, the latter being used by the microorganism to oxidize methane.

A Particular Case, the Anammox (*cf.* Sect. 14.3.5)

A new mode of use of nitrogen compounds was discovered among *Planctomycetes*, a group of bacteria with complex cell structure. The anammox process is an energy conservation based on the anaerobic oxidation of ammonium with nitrite as electron acceptor:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\,H_2O \qquad \Delta G^{\circ\prime} = -357\ \text{kJ}$$

Dissimilatory Sulfate Reduction

The sulfur compounds are important terminal electron acceptor for anaerobic respiration. Among them, sulfate, the most oxidized form of sulfur, is used as an electron acceptor by bacteria or archaea during a respiration called sulfate reduction. Sulfate-reducing microorganisms are generally strict anaerobes and produce sulfide as a toxic end product of their respiration. Other less oxidized sulfur compounds (sulfite, thiosulfate, sulfur, etc.) can also play the role of electron acceptors. Seeing the variety of electron donors and metabolic pathways that respiration of sulfur compounds involves, the development of a unified model of electron transfer is impossible with the exception of sulfate reduction steps. The reduction of sulfate to sulfide involves the transfer of eight electrons and the oxidation state of sulfur changes from + VI to − II:

$$SO_4^{2-} + 8\,H^+ + 8\,e^- \rightarrow S^{2-} + 4\,H_2O$$

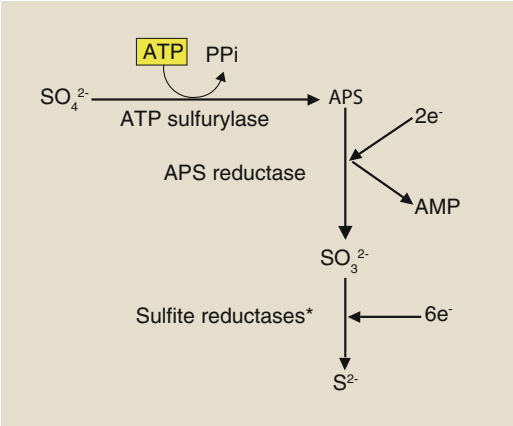

Fig. 3.22 Pathway of sulfate reduction. *APS* adenosine phosphosulfate, * 4 sulfite reductases known (desulfoviridin, desulforubidin, pigment P582, and desulfofuscidin), and *AMP* adenosine monophosphate (Drawing: M.-J. Bodiou)

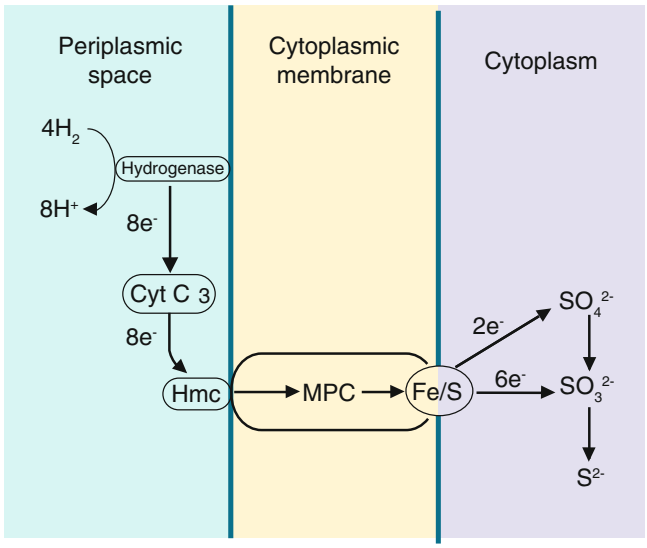

Fig. 3.23 Respiratory chain and formation of an H^+ gradient in *Desulfovibrio* from hydrogen as electron donor. *Cyt c3* cytochrome c3, *Hmc* high molecular weight cytochrome, *MPC* multiprotein complex, *Fe/S* iron–sulfur protein (Modified and redrawn from Voordouw 1995, Heidelberg et al. 2004, and Mathias et al. 2005) (Drawing: M.-J. Bodiou)

During this reduction, the sulfate must be activated by ATP. The activation reaction catalyzed by ATP sulfurylase leads to the formation of adenosine phosphosulfate (APS) (Fig. 3.22). The APS and sulfite accept electrons supplied by the respiratory chain.

The flow of electrons along the respiratory chain and the creation of a gradient of H^+ from molecular hydrogen oxidized by a periplasmic hydrogenase are shown in Fig. 3.23. The electrons are transferred to sulfur compounds by the APS reductase and sulfite reductase.

Table 3.9 Different genera of sulfate-reducing and sulfur-reducing microorganisms

Sulfate-reducing bacteria	**1. Complete oxidation**	Gram +	*Desulfotomaculum*
		Gram −	*Desulfobacter*
			Desulfococcus
			Desulfobacterium
			Desulfosarcina
			Desulfomonile
			Desulfonema
			Desulfoarculus
			Desulfacinum
			Desulforhabdus
	2. Incomplete oxidation	Gram +	*Desulfotomaculum*
		Gram −	*Desulfovibrio*
			Desulfomicrobium
			Desulfobulbus
			Desulfobotulus
			Desulfobacula
			Desulfofustis
Sulfate-reducing archaea			*Archaeoglobus*
Sulfur-reducing bacteria, non-sulfate reducing			*Desulfuromonas*
			Desulfurella
			Sulfurospirillum
			Wolinella
Sulfur-reducing archaea, non-sulfate reducing			*Pyrobaculum*
			Pyrodictium
			Pyrococcus
			Thermoproteus
			Thermophilum
			Thermococcus
			Desulfurococcus

With lactate as electron donor, the hydrogenases are also involved in the formation of a proton gradient.

Sulfate reducers are usually chemoorganotrophic microorganisms, but they are often chemolithotrophic microorganisms using dihydrogen as electron donor. The organic substrates used are mostly of small molecules (lactate, acetate, pyruvate, ethanol, propionate, butyrate, etc.) from fermentations of organic matter. Sulfate reducers are separated into two groups according to their ability to oxidize organic substrates, those which partially oxidize and those which completely oxidize to CO_2 (Table 3.9). Some are also able to oxidize some more complex substrates such as hydrocarbons (*cf.* Sect. 16.8.1), benzoate, phenol, starch, peptides, indole, sugars, amino acids, or glycerol.

During a reaction of syntrophy (*cf.* Sect. 14.3.3), sulfate reduction by sulfate-reducing bacteria is coupled to the

oxidation of methane by anaerobic methanotrophic archaea (reverse methanogenesis). Milucka et al. (2012) revealed the presence of methanotrophic archaea capable of coupling the anaerobic oxidation of methane to sulfate reduction; sulfate-reducing bacteria associated with methanotrophs get their energy from the **disproportionation*** of reduced sulfur compound (disulfide) released by methanotrophs.

The energy production is low, regardless of the substrates:

$$4H_2 + SO_4^{2-} \rightarrow S^{2-} + 4\,H_2O \qquad \Delta G^{\circ'} = -152\ \text{kJ}$$
$$2\ \text{lactate} + SO_4^{2-} \rightarrow S^{2-} + 2\ \text{acetate} + 2\,CO_2 + 2\,H_2O \qquad \Delta G^{\circ'} = -160\ \text{kJ}$$

ATP synthesis is the result of oxidative phosphorylation during operation of the respiratory chain; however, during the degradation of lactate by *Desulfovibrio*, an additional ATP synthesis occurs by phosphorylation at the substrate level:

$$\text{Lactate} \rightarrow \text{pyruvate} + 2\,e^- + 2\,H^+$$
$$\text{Pyruvate} + \text{ADP} + \text{Pi} \rightarrow \text{acetate} + CO_2 + \text{ATP} + 2\,e^- + 2\,H^+$$

During the oxidation of acetate to CO_2, the majority of sulfate reducers use the **inverse pathway of acetyl-CoA*** (inverse pathway of the acetogenesis). The pathway of the citric acid is specific to some sulfate-reducing bacteria (*Desulfobacter*, *Desulfuromonas*, *Desulfurella*). The sulfate reducers are heterotrophs, using small organic molecules, but some are facultative autotrophs and can fix CO_2 via the acetyl-CoA or via the reverse tricarboxylic acid cycle (*cf.* Sect. 3.4.1). The majority of sulfate reducers can also use sulfite, thiosulfate, and sulfur as electron acceptors. In addition, some are capable of thiosulfate and sulfite disproportionation, releasing sulfate and sulfide:

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + H_2S \qquad \Delta G^{\circ'} = -22\ \text{kJ}$$
$$4\,SO_3^{2-} + 2\,H^+ \rightarrow 3\,SO_4^{2-} + H_2S \qquad \Delta G^{\circ'} = -60\ \text{kJ}$$

The disproportionation of sulfur is thermodynamically unfavorable:

$$4\,S^\circ + 4\,H_2O \rightarrow SO_4^{2-} + 3\,H_2S + 2H^+ \qquad \Delta G^{\circ'} = +10\ \text{kJ}$$

However, if the sulfide is oxidized with the help of a metal (iron or manganese), the reaction is favorable:

$$3\,H_2S + 2\,\text{FeOOH} \rightarrow S^\circ + 2\,\text{FeS} + 4\,H_2O \qquad \Delta G^{\circ'} = -144\ \text{kJ}$$

Indeed, the sum of the two reactions above becomes

$$3S^\circ + 2\,\text{FeOOH} \rightarrow SO_4^{2-} + 2\,\text{FeS} + 2\,H^+ \qquad \Delta G^{\circ'} = -134\ \text{kJ}$$

In addition to sulfate reducers, there are sulfur-reducing microorganisms, reducing sulfur but unable to reduce sulfate. They are mostly found in *Archaea* (Table 3.9).

In the absence of sulfur compounds, sulfate-reducing bacteria can use other compounds as terminal electron acceptors. These are organic (fumarate, malate, organochlorines) or inorganic compounds (nitrate, derivatives of uranium, iron, selenium or arsenic, etc.) and more surprising the dioxygen for "anaerobic" bacteria. Research has shown that microorganisms belonging to the genus *Desulfovibrio* were particularly resistant to dioxygen (Le Gall and Xavier 1996). This is particularly true for those isolated from environments containing anoxic microniches or subjected to conditions of very fluctuating redox (interface zones, biofilms, microbial mats, etc.). Resistance enzymes to oxidative stress (catalase, superoxide dismutase, superoxide reductase) were found in some sulfate reducers. In addition, an oxygenase reductase has even been discovered, responsible for the dioxygen reduction by aerobic respiratory chains incompletely described in sulfate reducers (Santana 2008). In most cases, energy production by oxidative phosphorylation induced by the presence of dioxygen allows only the survival of sulfate reducers. However, the possibility of growth of *Desulfovibrio* has been partially demonstrated in partial pressure of dioxygen, close to that of the atmosphere (Lobo et al. 2007).

Ferric Iron Respiration or Ferric Reduction

The redox potential of the Fe^{3+}/Fe^{2+} couple (+770 mV) close to the potential of the O_2/H_2O couple (+820 mV) suggests a significant energy production during the respiration of iron (III). But, the very low solubility of Fe^{3+} ion at pH 7 (lower than 10^{-16} M) makes this process inefficient. However, anaerobic growth of *Shewanella oneidensis* and *Geobacter metallireducens* depends on the reduction of iron (III). This property is found in other bacteria (*Thermotoga*, *Thermus*, *Geothrix*, *Ferribacterium*, *Acidiphilum*) and archaea (*Pyrobaculum*). The link between microorganisms and insoluble iron oxides can be established by chelators (*Geothrix*), by direct contact with particles of iron oxides (*Geobacter*), or by the intermediate carriers called "shuttle" responsible for transfer electrons to insoluble iron (*Geobacter*). Humic substances widespread in natural environments can play the role of shuttles. Indeed, humic substances contain quinone groups that can undergo oxidation–reduction cycles; microorganisms transfer electrons to humic substances that oxidize again in contact with particles of iron (III) (*cf.* Sect. 14.5.2). Bacteria that use iron as a terminal electron acceptor is usually facultative anaerobic chemoorganotrophic bacteria except of some strict anaerobes (*Geobacter metallireducens*). They use various organic substrates as electron donors and carbon sources. Their activity is limited to interfaces between oxic and anoxic environments where anaerobiosis and the presence of iron (III) can coexist (*cf.* Sect. 14.5.2).

Fumarate Respiration

Fumarate is not abundant in natural environments. However, fumarate respiration is widespread among microorganisms, probably due to the fact that fumarate is a common metabolite, formed from the catabolism of carbohydrates and proteins. This type of respiration is known in *Wolinella succinogenes*, *Enterobacteriaceae*, *Clostridia*, *Paenibacillus macerans*, sulfate-reducing bacteria, and *Propionibacteria*.

The reducing power resulting from the oxidation electron donors, mainly hydrogen, formate, or NADH, is transferred by the respiratory chain to a fumarate reductase which reduces the fumarate to succinate:

$$H_2 + \text{fumarate} \rightarrow \text{succinate}$$
$$\text{Formiate} + \text{fumarate} + H^+ \rightarrow CO_2 + \text{succinate}$$

With a redox potential of + 30 mV, the fumarate/succinate couple is not the source of important production of energy. For example, the first reaction above produces only -43 kJ.mol^{-1} dihydrogen and therefore will require more than one mole of dihydrogen to synthesize one mole of ATP.

In addition to the fumarate, other organic molecules can play the role of electron acceptors during anaerobic respirations such as glycine, dimethyl sulfoxide, and trimethylamine oxide reduced, respectively, in acetate, dimethyl sulfide, or trimethylamine.

CO_2 Respirations (Acetogenesis and Methanogenesis)

CO_2 from metabolism of chemoorganotrophic microorganisms is an abundant compound in natural environments and serves as a terminal electron acceptor for two groups of strict anaerobic microorganisms, acetogenic bacteria and methanogenic archaea. In anoxic environments, these microorganisms have at their disposal electron donors from the decomposition of organic matter, in particular dihydrogen. Thus, some of them are chemolithotrophic autotrophs. CO_2 reduction leads to the formation of acetate in acetogens and methane in methanogens. Redox couples are very electronegative (CO_2/CH_4, -0.24 V and CO_2/acetate, -0.29 V); consequently, the two respirations are low in energy:

$$4\,H_2 + CO_2 \rightarrow CH_4 + 2\,H_2O \qquad \Delta G^{\circ'} = -131 \text{ kJ}$$
$$4\,H_2 + 2\,CO_2 \rightarrow CH_3COOH + 2\,H_2O \qquad \Delta G^{\circ'} = -95 \text{ kJ}$$

– *Acetogenesis*

Acetogenic bacteria form a very heterogeneous group of Gram-positive bacteria essentially (*Acetobacterium*, *Butyribacterium*, *Clostridium*, *Eubacterium*, *Moorella*, *Sporomusa*). They use CO_2, CO, or formate as electron acceptors and produce acetate as an end product of their respiration. The electron donor is mostly dihydrogen. However, molecules such as sugars, alcohols, organic acids, or aromatic compounds can serve as electron donors and carbon sources. In all cases, the reduction of CO_2 passes through acetyl-CoA (Wood–Ljungdahl pathway) (*cf.* Sect. 3.4.1).

Table 3.10 Main metabolic pathways of methanogenic archaea

Metabolic reactions	$\Delta G^{\circ'}$ in kJ/mole of CH_4
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	-131
$H_2 + CH_3OH \rightarrow CH_4 + H_2O$	-112
$2CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2CH_3COO^- + 2H^+$	-116
$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$	-450
$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	-130
$4(CH_3)_3NH^+ + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_4^+$	-76
$CH_3COOH \rightarrow CH_4 + CO_2$	-36

With dihydrogen as electron donor, the reduction of one molecule of CO_2 as a methyl group and the reduction of a second molecule of CO_2 in the form of carbonyl function lead to the formation of acetyl-CoA in the presence of coenzyme A; acetyl-CoA is subsequently phosphorylated to acetyl phosphate which releases a molecule acetate and an ATP molecule (substrate-level phosphorylation).

During this process, energy can also be produced by the formation of a Na^+ gradient, instead of H^+ gradient, at the origin of a sodium-motive force (Muller 2003; Detkova and Pusheva 2006).

– *Methanogenesis*

All organisms are methanogenic archaea. Methanogenesis with dihydrogen as substrate and CO_2 as electron acceptor and carbon source is very widespread among the methanogenic microorganisms. However, other compounds involving other pathways could be used by some methanogens. Methanogenic reactions can be divided into two groups (Table 3.10). In the first group, methanogenesis involves the reduction of C1 molecules (CO, CO_2, and methanol) with dihydrogen or alcohols having more than one carbon atom as electron donors. The second group relates to microorganisms carrying out disproportionation reactions of compounds in C1 (CO, formate, formaldehyde, and methanol), methylamines, and methylsulfide (different genera of methanogens) or acetate (*Methanosarcina* and *Methanothrix*). During these reactions, a fraction of the compounds is oxidized to CO_2 and the other is reduced to CH_4 (*cf.* Sect. 14.2.6).

Of all the ways, the reduction of CO_2 with dihydrogen as electron donor is the best known. This reaction requires a series of specific coenzymes, carriers of carbon groups, particularly the coenzyme M (CoM-SH).

Conservation of energy is coupled to the reduction of the disulfide bridge of heterodisulfide (CoB-SS-CoM). Figure 3.24 shows a representation of mechanism of energy conservation in a methanogen leading to the formation of a proton gradient allowing energy production. The electrons

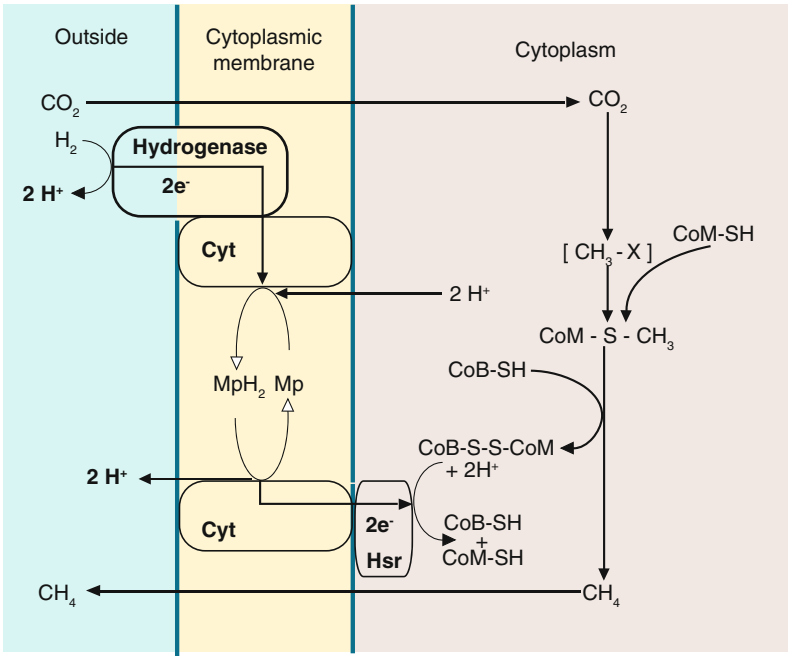

Fig. 3.24 Pathway of CO_2 reduction to CH_4 (CO_2 respiration) and formation of the membrane potential in a methanogenic (Modified and redrawn from Deppenmeier et al. 1999). CO_2 is successively reduced in formyl, methenyl, methylene, and methyl groups which are linked to coenzymes acting as carriers of carbon groups, and finally CH_4 released during the formation of heterodisulfide (CoB-SS-CoM). *[CH_3-X]* CH_3 bound, *CoM-SH* coenzyme M, *HS-CoB* coenzyme B, *Cyt* cytochrome, *Mp* oxidized methanophenazine, *MpH_2* reduced methanophenazine, and *Hsr* heterodisulfide reductase (Drawing: M.-J. Bodiou)

from the oxidation of dihydrogen are transferred by the respiratory chain to a reductase which reduces the disulfide bridge, thus releasing the CoM-SH available once again for the final reduction of CO_2. H^+ gradient is created by the oxidation–reduction of an intermediary carrier, the methanophenazine. In addition, during the CO_2 reduction cycle to CH_4, a sodium pump is activated and generates a sodium gradient allowing a sodium-motive force (Deppenmeier et al. 1999; Muller et al. 2008).

Methanogenic pathways using other compounds (methanol, acetate) are different, but some carriers are common.

3.3.3 Fermentations

In the absence of light, dioxygen, and other extracellular acceptors of electrons, the energy required for various cellular activities can be provided by fermentations. Pasteur has defined fermentation as "la vie sans air" ("life without air"). This definition is now not accurate. Mechanisms of energy producers that are not fermentations occur in the absence of air (anaerobic respirations, anoxygenic photosynthesis), and certain fermentations occur in the presence of air (e.g., lactic fermentation). A more accurate definition might be as follows: fermentations are energy producer mechanisms occurring usually under anaerobic conditions, energy being conserved mainly by substrate-level phosphorylation. Electron donors are organic compounds. Electron acceptors are formed by endogenous organic compounds obtained from cellular metabolism and derived from the partial oxidation of electron donors. Unlike process of aerobic and anaerobic respirations, in fermentations there is no exogenous electron acceptor, with the exception of the fermentation of some amino acids which require an amino acid that acts as a donor of electrons and an amino acid that plays the role of electron acceptor (Stickland reaction). In the majority of fermentations, energy production does not involve a chain of membrane electron carriers; redox processes take place into the cytoplasm.

While in most respirations, the electron donor is completely oxidized to CO_2, in fermentations, the electron donor is only partially oxidized and thus provides fermentation products (organic acids, alcohols, etc.) that are released into the external environment and that often characterize the type of fermentation. A practical consequence of this excretion of products is the use of fermentations in the manufacture of foods and products for food, pharmaceutical, or chemical industries. The reducing power is temporarily transferred to coenzymes (NAD^+ in general) which oxidize again by transferring electrons to an organic compound (which serves as a final electron acceptor) originating from the oxidation pathway of the electron donor (Fig. 3.25). The main fermentation products are CO_2, dihydrogen, formate, acetate, lactate, and short-chain fatty acids. Ammonium, sulfide, methyl mercaptans, and aromatic compounds come from the fermentation of amino acids.

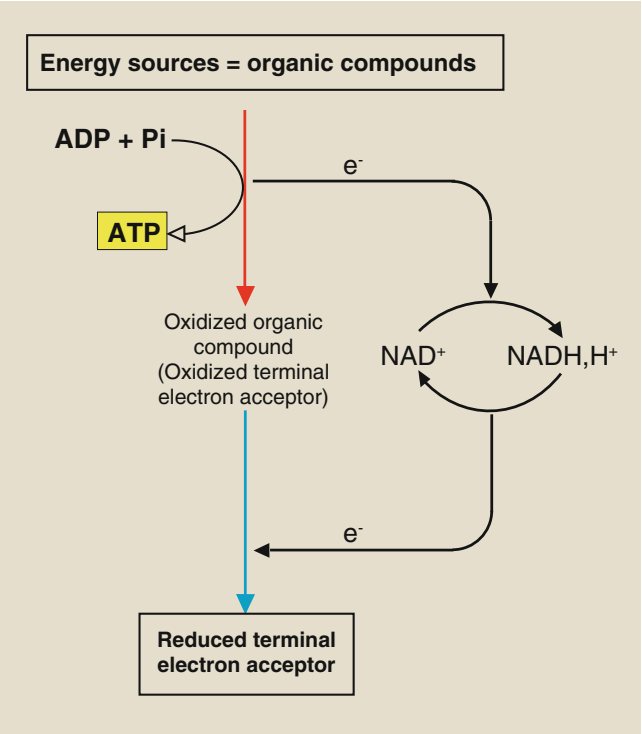

Fig. 3.25 General scheme of fermentation (Drawing: M.-J. Bodiou)

3.3.3.1 Dihydrogen Production by Fermentations

Many fermentation processes produce dihydrogen to maintain their redox balance. Hydrogenase or a formate hydrogen lyase catalyzes the formation of dihydrogen:

$$\text{Pyruvate} \rightarrow e^- \rightarrow \text{ferredoxin} \rightarrow 2H^+ \rightarrow H_2(\text{hydrogenase})$$
$$\text{Formate} \rightarrow CO_2 + H_2(\text{formate hydrogen lyase})$$
$$NADH, H^+ \rightarrow e^- \rightarrow \text{ferredoxin} \rightarrow 2H^+ \rightarrow H_2(\text{hydrogenase})$$

The third reaction thermodynamically unfavorable can take place only during interspecies dihydrogen transfer.

3.3.3.2 Mechanism of Energy Conservation

ATP is synthesized by substrate-level phosphorylation. For the same substrate, the fermentation is much less efficient than respiration. For example, for one mole of glucose, respiration in yeast produces 2,872 kJ and alcoholic fermentation only 236 kJ:

$$C_6H_{12}O6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad \Delta G^{\circ'} = -2,872\ \text{kJ.mole}^{-1}$$
$$C_6H_{12}O6 \rightarrow 2C_2H_6O + 2CO_2 \quad \Delta G^{\circ'} = -236\ \text{kJ.mole}^{-1}$$

With a free energy of −236 kJ per mole of glucose fermented, several moles of ATP should be produced. In fact, the alcoholic fermentation releases only two moles of ATP.

This low efficiency, which characterizes all fermentations, obliges the fermentative microorganisms to degrade large amounts of substrate and thus produce large quantities of products used in biotechnology.

Among the many reactions of substrate-level phosphorylation, three are frequent during the fermentation of carbohydrates:

$$1,3 - \text{diphosphoglycerate} + \text{ADP} \rightarrow 3 - \text{phosphoglycerate} + \text{ATP}$$
$$\text{Phosphoenolpyruvate} + \text{ADP} \rightarrow \text{pyruvate} + \text{ATP}$$
$$\text{Acetyl phosphate} + \text{ADP} \rightarrow \text{acetate} + \text{ATP}$$

In addition to phosphorylation at the substrate level, there are, in some fermentative bacteria, other mechanisms of energy conservation:

1. During the propionic fermentation by *Propionibacterium*, in addition to the phosphorylation at the substrate level, the bacterium uses the formation of membrane gradients of H^+ and Na^+ (proton- and sodium-motive forces). The fermentative pathway is complex and involves the formation of C4 dicarboxylic acids, leading to the reduction of fumarate to succinate via a chain of electron carriers. This transfer is coupled to proton translocation. Then the succinate is decarboxylated under the action of a membrane decarboxylase resulting in Na^+ excretion.
2. During the malolactic fermentation, malate enters into the cell as a di-anion via a permease which exchanges it with a lactate mono-anion. This ionic imbalance which is equivalent to an output of H^+ generates a proton-motive force.

Table 3.11 The major fermentations

Fermented substrates	Names of fermentations	Fermentative products	Examples of microorganisms
Sugars	Alcoholic	Ethanol, CO_2	Yeasts, *Zymomonas*
	Lactic	Lactic acid and sometimes ethanol, acetic acid, CO_2	*Lactobacillus*, *Lactococcus*, *Leuconostoc*
	Butyric	Butyric acid, acetic acid, CO_2, H_2	*Clostridium*, *Butyribacterium*
	Acetonobutylic	Acetone, butanol, CO_2, H_2	*Clostridium acetobutylicum*
	Acetic	Acetic acid	*Clostridium thermoaceticum*
	Mixed acids	Formic acid, acetic acid, lactic acid, succinic acid, ethanol, CO_2, H_2	*Escherichia*, *Salmonella*, *Shigella*, *Proteus*
	2,3-Butanediol	2,3-Butanediol, lactic acid, formic acid, ethanol, CO_2, H_2	*Enterobacter*, *Serratia*, *Erwinia*
	Propionic	Propionic acid, acetic acid, CO_2	*Propionibacterium*, *Corynebacterium*
Organic acids			
Lactic acid	Propionic	Propionic acid, CO_2	*Clostridium propionicum*
Malic acid	Malolactic	Lactic acid, CO_2	*Leuconostoc oenos*
Citric acid		Acetoin, diacetyl, acetic acid, lactic acid, CO_2	*Lactococcus cremoris*, *Leuconostoc cremoris*
Amino acids			
Alanine		Propionic acid, acetic acid, NH_3, CO_2	*Clostridium propionicum*
Glycine		Acetic acid, NH_3, CO_2	*Peptococcus anaerobius*
Threonine		Propionic acid, NH_3, H_2, CO_2	*Clostridium propionicum*
Arginine		Ornithine, CO_2, NH_3	*Clostridium*, *Streptococcus*
Cysteine		H_2S, NH_3, pyruvic acid	*Proteus*, *Escherichia*, *Propionibacterium*
Tryptophan		Indole propionic acid, indole pyruvic acid, NH_3	*Clostridium sporogenes*
		Indole, pyruvate, NH_3	*Escherichia coli*
Heterocyclic compounds			
Guanine, xanthine		Glycine, formic acid, NH_3, acetic acid, CO_2	*Clostridium cylindrosporum*
Urate		Acetic acid, CO_2, NH_3	*Clostridium acidurici*

3. In homolactic bacteria, excretion of lactate takes place by symport with H^+ forming a proton gradient.

These different processes which concern only special cases in fermentations allow an additional production of energy.

3.3.3.3 Diversity of Fermentations and Their Metabolic Pathways

Fermentations are classified according to the formed products and consumed substrates (Table 3.11). Very widespread among microorganisms, the fermentative processes are very diverse. Microorganisms may be facultative fermentative microorganisms (also capable of aerobic or anaerobic respirations) or obligate fermentative microorganisms, and in this case either anaerobic or **air tolerant***.

The group of prokaryotes contains the majority of fermentative microorganisms. However, fermentative pathways are known in some eukaryotes. Besides yeasts, some fungi (*Anaeromyces*, *Neocallimastix*, *Orpinomyces*, etc., present in the digestive tract of herbivorous) possess a fermentative metabolism. Some protozoa, parasites (*Giardia*, *Entamoeba*, *Trichomonas*, etc.), as well as commensal or symbiotic (*Dasytricha*, *Isotricha*, *Trichonympha*) and free protozoa (*Hexamita*, *Trimyema*) can obtain the energy necessary to their activity during fermentation. Many of these fungi and protozoa lack mitochondria but have particular organelles, hydrogenosomes, for the origin of dihydrogen and ATP productions (*cf.* Sects. 4.3.4 and 5.4.2).

Many metabolic pathways have been described in prokaryotes, particularly in bacteria. During fermentation, sugars are generally oxidized to pyruvate which can be considered the main key to fermentative metabolism of sugars (Fig. 3.26). It is the source of various fermentation products resulting from the use of different pathways. Pyruvate or its degradation products become electron acceptors to allow oxidation of reduced coenzymes. Pyruvate is also a fermentative intermediate for other substrates but is not obligatory intermediate for all fermentations. Table 3.12 shows the equations and the productions of free energy of some fermentations.

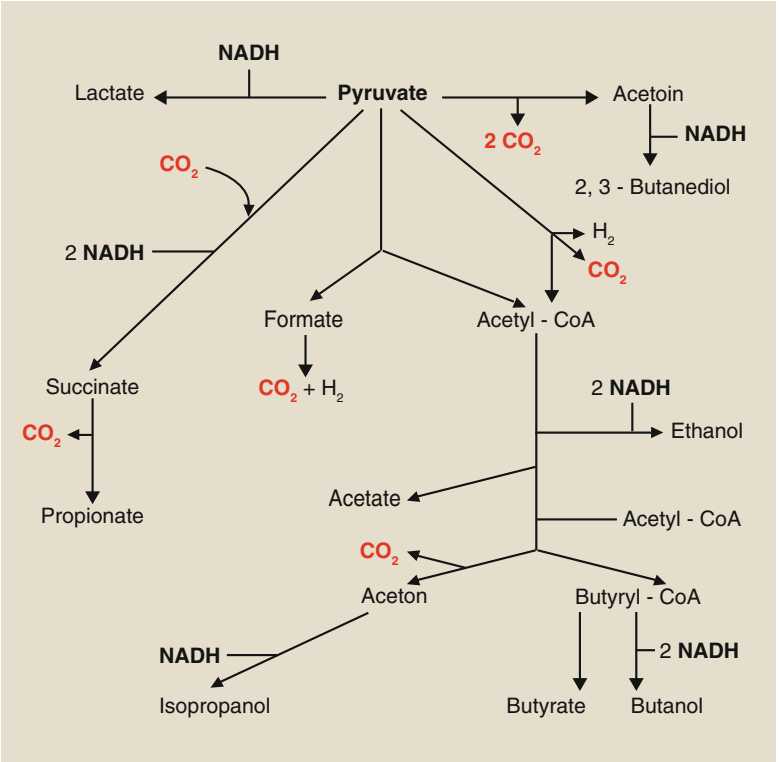

Fig. 3.26 Major pathways of reduction of pyruvate in the fermentation (Modified and redrawn from Stanier et al. 1986. Drawing: M.-J. Bodiou)

Table 3.12 Examples of metabolic reactions in some fermentations

Fermentations	Equations of fermentations	$\Delta G^{\circ\prime}$ in kJ/reaction	Examples of microorganisms
Alcoholic	Glucose → 2 ethanol + 2 CO_2	−236	Yeasts, *Zymomonas*
Homolactic	Glucose → 2 lactate	−198	*Lactobacillus*
Heterolactic	Glucose → lactate + ethanol + CO_2	−177	*Lactobacillus*
	Ribose → lactate + acetate	−210	*Leuconostoc*
Butyric acid	Glucose → butyrate + 2 CO_2 + 2 H_2	−224	*Clostridium*
Malolactic	Malate → lactate + CO_2	−67.3	*Leuconostoc*
Propionic acid	3 lactate → 2 propionate + acetate + CO_2	−170	*Propionibacterium*
Alanine	3 alanine + 2H_2O → 3 NH_3 + CO_2 + 3 acetate + 2 propionate	−135	*Clostridium*
Alanine (Stickland reaction)	Alanine + 2 glycine + 2 H_2O → 3 acetate + CO_2 + 3 NH_3	−107	*Clostridium*
Glutamate	5 glutamate + 6 H_2O → 5 NH_3 + 5 CO_2 + 6 acetate + 2 butyrate + H_2	−300	*Clostridium*
Glycine	4 glycine + 2 H_2O → 4 NH_3 + 2 CO_2 + 3 acetate	−217	*Eubacterium*

3.3.3.4 Syntrophy Interspecies Hydrogen Transfer

During fermentation, the reaction of **syntrophy*** or interspecies hydrogen transfer is a mechanism involving two microorganisms in anoxic conditions: a first microorganism that ferments a low fermentable substrate (propionate, butyrate, ethanol) producing acetate and dihydrogen (syntrophic microorganism: *Syntrophobacter*, *Syntrophomonas*), only in the presence of a second microorganism that consumes the dihydrogen as it is produced. The direct reaction of fermentation of these poorly fermentable substrates by the syntrophic microorganism is impossible because it is thermodynamically unfavorable. The reaction requires energy and therefore cannot be coupled to a mechanism that generates energy. This reaction is only possible if the dihydrogen produced during fermentation is maintained at a very low level (partial pressure of about 10^{-4} atm or less) by the activity of a second microorganism that consumes dihydrogen as soon as it is produced; the main

Table 3.13 Fermentative reactions in syntrophic microorganisms

Reactions of syntrophic metabolism	$\Delta G^{\circ\prime}$ in kJ	Syntrophic microorganisms
Butyrate + $2H_2O \rightarrow$ 2 acetate + $H^+ + 2H_2$	+48.1	*Syntrophomonas*
Propionate + $2H_2O \rightarrow$ acetate + $3H_2 + CO_2$	+71.3	*Syntrophobacter*
Lactate + $2H_2O \rightarrow$ acetate + $2\ H_2 + CO_2$	−9	*Desulfovibrio*
$CH_4 + 2\ H_2O \rightarrow CO_2 + 4\ H_2$	+130	Methanotrophic archaea (reverse methanogenesis)

microorganisms that consume dihydrogen are sulfate-reducing bacteria, acetogenic bacteria, or methanogenic archaea. This interspecies hydrogen transfer reaction is called syntrophic reaction because both microorganisms involved acquire a benefit from their interrelationship (Stams and Plugge 2009).

A well-known case of syntrophy is the fermentation of ethanol to acetate and methane by a coculture of a syntrophic bacterium and a methanogenic archaea. The syntrophic bacterium ferments ethanol to acetate and dihydrogen:

$$2\ \text{ethanol} + 2\ H_2O \rightarrow 2\ \text{acetate} + 4\ H_2 + 2\ H^+ \quad \Delta G^{\circ\prime} = +20\ \text{kJ}$$

The endergonic reaction cannot allow the development of this bacterium unless the dihydrogen partial pressure is lowered to 10^{-4} atm. Only in this case, the fermentation by the syntrophic bacteria becomes exergonic and releases energy (−44 kJ) for its growth (Zehnder and Stumm 1988). The methanogenic archaea consume dihydrogen and thus the syntrophic bacteria produces energy and can grow. The resultant of the two equations is in favor of energy production:
Syntrophic bacterium:

$$2\ \text{ethanol} + 2\ H_2O \rightarrow 2\ \text{acetate} + 4\ H_2 + 2\ H^+ \quad \Delta G^{\circ\prime} = +20\ \text{kJ}$$

Methanogenic archaea:

$$4H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O \quad \Delta G^{\circ\prime} = -131\ \text{kJ}$$

Results:

$$2\ \text{ethanol} + CO_2 \rightarrow 2\ \text{acetate} + CH_4 + 2H^+ \quad \Delta G^{\circ\prime} = -111\ \text{kJ}$$

In Table 3.13, some examples of possible syntrophic fermentations by interspecies hydrogen transfers are presented.

3.3.4 Photosynthesis

Phototrophic microorganisms (also called photosynthetic microorganisms) use solar energy as an energy source that they capture and convert into chemical energy. Solar energy becomes thus available for the different cellular activities and particularly for the synthesis of ATP and the reduction of coenzymes (NAD^+ and $NADP^+$). Phototrophic microorganisms in their great majority autotrophs (photoautotrophic microorganisms) use these coenzymes to reduce CO_2 into organic compounds. This synthesis of organic compounds with light as an energy source is called photosynthesis. Photosynthesis occurs in two phases: a light phase during which light energy is converted into chemical energy producing ATP and reduced coenzymes, followed by a dark phase of CO_2 reduction and synthesis of organic compounds that consumes ATP and reduced coenzymes. Photosynthesis depends on the presence of photosynthetic pigments, in particular of chlorophylls. However, there are specific cases where the mechanism of transformation of light energy into chemical energy is independent of chlorophylls and depends on totally different types of pigments (e.g., bacteriorhodopsin) especially in some extreme halophilic archaea. Photosynthesis is the most important metabolic processes for life on our planet at the base of most food chains; it allows the introduction of the energy which is necessary for the preservation of life into the terrestrial biosystem.

In chlorophyll microorganisms, the presence or absence of a release of dioxygen during photosynthesis subdivides microorganisms into oxygenic phototrophs and anoxygenic phototrophs.

In oxygenic phototrophic microorganisms that include oxygenic phototrophic bacteria (cyanobacteria) and photosynthetic eukaryotes, electron donor for the reduction of coenzymes is water, and photosynthesis is accompanied by dioxygen evolution. In anoxygenic phototrophic microorganisms that are all of the bacterial domain, the electron donors are more reduced than water. They can be organic compounds of low molecular weight which also serve as carbon source (anoxygenic photoorganotrophic and photoheterotrophic bacteria) or inorganic compounds such as reduced sulfur compounds, dihydrogen, or sometimes the ferrous iron (anoxygenic photolithotrophic bacteria) (*cf.* Sects. 14.4.3 and 14.5.2). These photolithotrophic bacteria use CO_2 as a carbon source and thus are photoautotrophic bacteria (Table 3.14).

3.3.4.1 Photosynthetic Pigments of Chlorophyll-Containing Phototrophic Microorganisms

The chlorophylls (Chls) of oxygenic phototrophic microorganisms and bacteriochlorophylls (BChls) of anoxygenic phototrophic bacteria consist of a porphyrin core containing in its center a magnesium atom. Five chlorophylls and six bacteriochlorophylls are known. They differ in the nature of the substituents connected to the

Table 3.14 Main characteristics of chlorophyll-containing phototrophic microorganisms

	Electron donors	Carbon source	Phototrophic types
Oxygenic phototrophic microorganisms			
Photosynthetic microeukaryotes	H_2O	CO_2	Photolithotroph, photoautotroph
Cyanobacteria	H_2O	CO_2	Photolithotroph, photoautotroph
Anoxygenic phototrophic bacteria			
Purple and green sulfur bacteria	H_2S, S°, $S_2O_3^{2-}$, Fe^{2+}, H_2	CO_2	Photolithotroph, photoautotroph
Purple and green nonsulfur bacteria	Organic compounds	Organic compounds	Photoorganotroph, photoheterotroph

Table 3.15 Wavelengths of light absorption of different chlorophylls (Chl) and bacteriochlorophylls (Bchl) in whole cells

Chlorophylls	Wavelengths of light absorption
Chl *a*	430–440, 670–675
Chl *b*	480, 650
Chl *c* (c1, c2, c3)	450, 630
Chl *d*	440, 670, 718
Divinyl-chlorophyll *a*[a]	440, 660
BChl *a*	375, 590, 805, 830–920
BChl *b*	400, 605, 835–850, 1020–1040
BChl *c*	460, 745–755
BChl *d*	450, 715–745
BChl *e*	460, 710–725
BChl *g*	375, 419, 575, 670, 788

[a]Divinyl-chlorophyll is present in prochlorophytes
Chl chlorophyll, *BChl* bacteriochlorophyll

periphery of the porphyrin. In vivo, Chls and BChls form complexes with proteins that absorb light at different characteristic wavelengths (λ) (Table 3.15).

The absorption maxima are complementary: photosynthesis in microorganisms is active in a wide band of the solar spectrum from 400 to 1,040 nm. Other pigments associated with chlorophylls also trap light in phototrophic microorganisms. The most common are phycobiliproteins (phycocyanin and phycoerythrin) of cyanobacteria and carotenoid pigments. They absorb light radiations between 450 and 650 nm, region where Chls and BChls absorb little or no light. They allow better utilization of wavelengths and protect the cell and the Chls and BChls from the photooxidation reactions. Abundant carotenoids mask the color of the BChls. In anoxygenic phototrophic bacteria called "purple bacteria" (e.g., *Chromatium*, *Rhodospirillum*, etc.) that have BChl *a* or *b*, carotenoid pigments give them colors ranging from yellow-orange, pink, red, brown to purple-violet. Anoxygenic phototrophic bacteria that are qualified of phototrophic green bacteria (e.g., *Chlorobium*, *Chloroflexus*, etc.) which possess BChl *c*, *d*, or *e* and specific carotenoids in small amounts reveal cell suspension colors from yellow-green to green-brown (*cf.* Sect. 14.4.3; Fig. 14.39). The combination of chlorophyll and phycocyanin gives a blue-green color to cells of cyanobacteria. This color is the origin of the old name given to these prokaryotic microorganisms (Cyanophyceae, Cyanophyta, or blue-green algae). Some cyanobacteria that contain phycoerythrin have cell colors ranging from pink to red. According to the microorganisms, the pigments are combined in different antenna or light-harvesting molecules that are responsible for transferring the light energy to a reaction center (RC) where the transformation of the light energy into chemical energy occurs. Only a very small number of Chl *a* molecules are present in the reaction centers of oxygenic phototrophic microorganisms and, usually, Bchl *a* molecules in the reaction centers of phototrophic bacteria, either purple or green. These molecules associated with electron transport chains are inserted into specialized membranes, generating a proton-motive force and allowing the reduction of coenzymes.

The intracellular location of antenna and RC depends on the type of phototrophic microorganism (Fig. 3.27). In photosynthetic microeukaryotes, all pigments are included in the specialized membrane systems (thylakoids) within chloroplasts. In cyanobacteria (prokaryotic microorganisms), the thylakoids are directly present into the cytoplasm and contain the reaction centers in their membrane, while the antennae pigments form aggregates on their surface. In anoxygenic phototrophic purple bacteria, all the pigments are inserted into the cytoplasmic membrane that develops and forms characteristic invaginations like lamellae, tubules, vesicles, etc. In anoxygenic phototrophic green bacteria, the antennae are located into ovoid structures (chlorosomes) attached to the inner surface of the cytoplasmic membrane, while the associated RCs are inserted directly in the cytoplasmic membrane.

3.3.4.2 Oxygenic Photosynthesis (Fig. 3.28a, b)

The reaction center (RC) of phototrophic eukaryotic microorganisms and cyanobacteria is incorporated into photosystems included in the thylakoid membrane. A photosystem comprises an antenna complex and a reaction center which is associated with a chain of electron carriers.

RCs are composed of proteins, Chl *a*, and initial electron acceptors that convert photon energy into chemical energy. Two coupled photosystems are required. In the photosystem I, the reaction center has a Chl *a* dimer which absorbs light at 700 nm (P700), whereas in the photosystem II, the dimer is active at a wavelength of 680 nm (P680). In general, the two

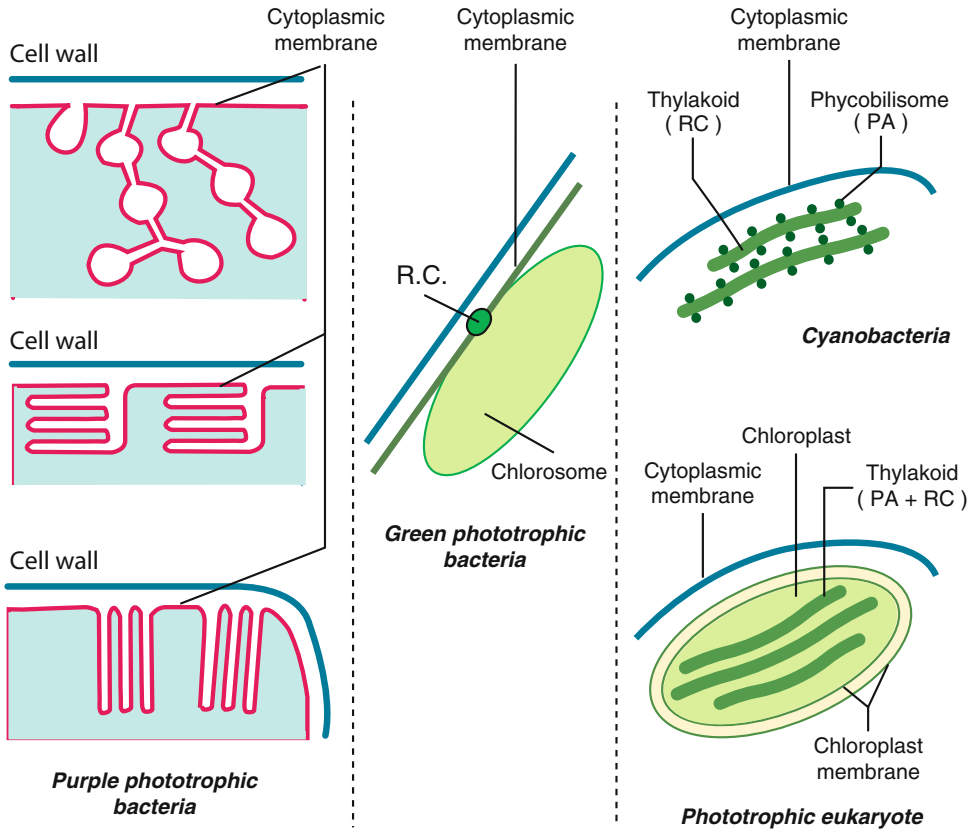

Fig. 3.27 Cellular localization of pigments in phototrophic microorganisms. *AP* antenna pigment; *RC* reaction center (Drawing: M.-J. Bodiou)

photosystems operate simultaneously; electrons are transferred from photosystem II to photosystem I.

By absorbing a photon, the P680 dimer of photosystem II passes from a ground state to an excited state which is strongly reduced, allowing the transfer of electron along the first chain of electron carriers to the RC of photosystem I (P 700). For this, P680 dimer gives its electron very quickly to a pheophytin (Chl molecule devoid of magnesium) at low redox potential which transfers it to the chain of electron carriers. A new light excitement transfers another electron and so on. The electrons are used for reducing the dimer P700 when excited by light. This transfer is accompanied by a translocation of protons in the center of the thylakoid to the origin of a proton-motive force that generates ATP by photophosphorylation via ATP synthases of the thylakoid. Chl of photosystem II, which has lost electrons during its excitation by light, recovers electrons from a water molecule. The $Chl^{oxdized}$/Chl couple having a redox potential higher than O_2/H_2O redox couple (+0.9 and +0.82 volts, respectively) gives this reaction thermodynamically possible. The oxidation of the water molecule by loss of electrons is accompanied by release of dioxygen. The protons released contribute to the formation of the proton gradient at the level of the thylakoid membrane:

$$H_2O \rightarrow 2\,e^- + 2\,H^+ + \tfrac{1}{2}\,O_2$$

In photosystem I, the electron of the dimer of Chl *a* (P700) excited by a photon is transferred to a first carrier A0 (Chl *a* modified) and then a second A1. Then the electrons pass through a chain of carriers containing iron sulfur protein and ferredoxin. Finally, the electrons are transferred to NADP reductase that reduces the coenzyme. The reduction of the coenzyme with water as electron donor is thermodynamically unfavorable:

$$NADP^+ + H_2O \rightarrow NADPH, H^+ + \tfrac{1}{2}\,O_2 \quad \Delta G^{\circ'} = +220\ kJ/NADP^+$$

Both activation steps are needed to bring the electrons at the top of photosystem I which represents the energy change required for efficient transfer of electrons from water to $NADP^+$. This flux of electrons in one direction is noncyclic transport or noncyclic photophosphorylation which is also called schema "Z."

When the reducing power (NADPH, H^+) is sufficiently large, cyclic electron transfer or cyclic photophosphorylation takes place involving only photosystem I. The electrons transit directly from Fd to complex b/f to reduce the P700 Chl. This transfer creates a proton gradient for ATP formation.

3.3.4.3 Anoxygenic Photosynthesis (Fig. 3.29a–c)

Anoxygenic phototrophic bacteria have only one photosystem. Photosystem of phototrophic purple bacteria is

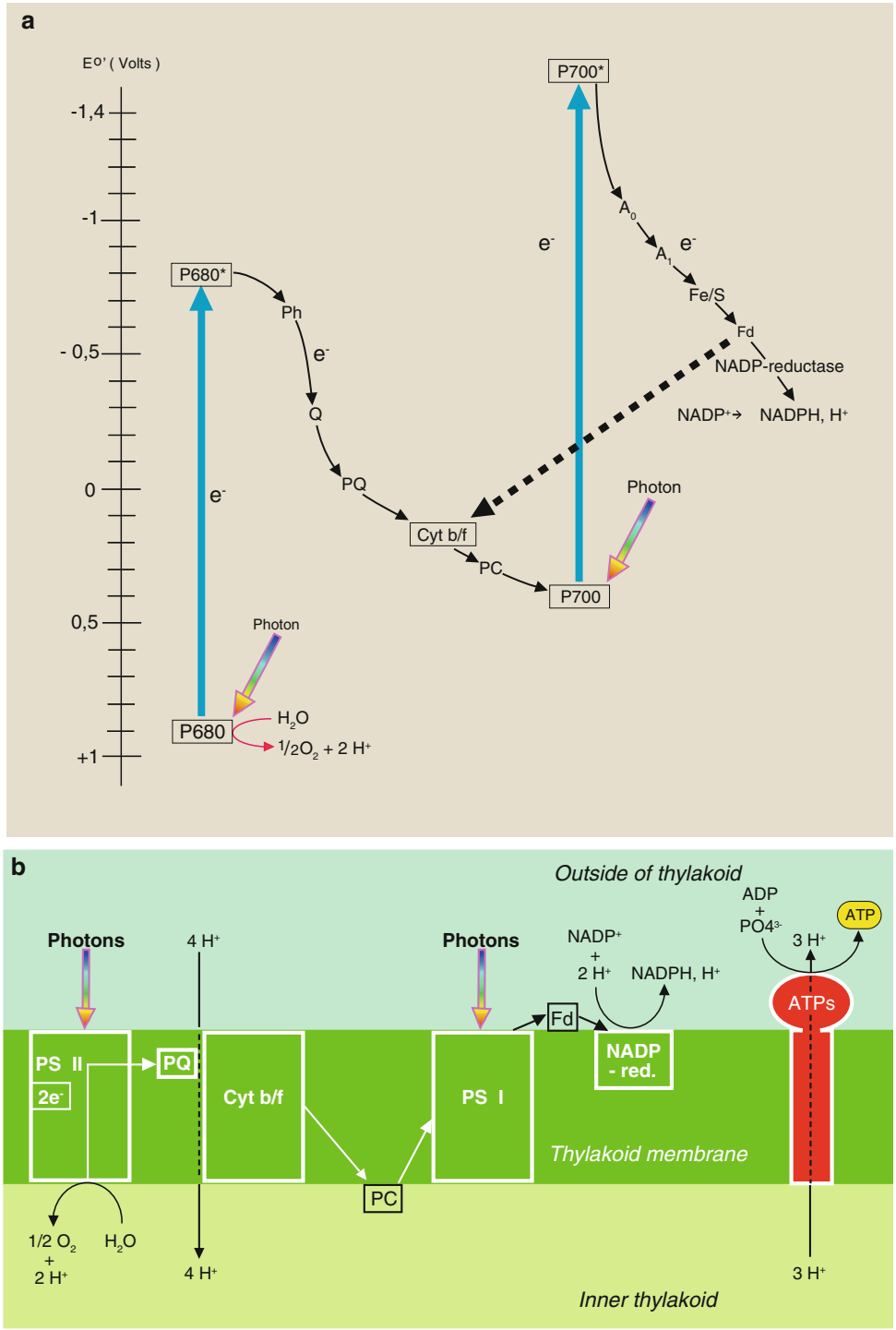

Fig. 3.28 Schemes of oxygenic photosynthesis. (**a**) The Z scheme of electron flow. *P680 and P700* chlorophyll of reaction centers of photosystems II and I, respectively, *P680* and P700**: excited chlorophylls, *Ph* pheophytin, *Q* quinone, *PQ* plastoquinone (substituted quinones), *Cyt b/f* cytochromes b and f complex, *Pc* plastocyanin (copper protein), *A0 and A1* electron acceptors (A 0, chlorophyll amended), Fe/S iron–sulfur protein, and *Fd* ferredoxin (iron–sulfur protein). (**b**) Location of the photosynthetic apparatus in the thylakoid membrane. *PS I and PS II* photosystems I and II, respectively, *PQ* plastoquinones, *PC* plastocyanin, *Fd* ferredoxin, *NADP-red* NADP reductase, and *ATPs* ATP synthase. The complex Cyt b/f plays the role of proton pump (Drawing: M.-J. Bodiou)

similar to photosystem II, while that of phototrophic green bacteria is similar to photosystem I. Reaction centers are included in the normal cytoplasmic membrane in green bacteria or in invaginated cytoplasmic membrane in purple bacteria. Because there is only one photosystem, the electron transfer is cyclic (cyclic photophosphorylation) and allows the production of chemical energy necessary for cellular activities. Noncyclic transfer is necessary for the reduction of coenzymes. This transfer requires energy and a reverse flux of electrons for purple bacteria.

The redox potential of BChl *a* which is involved in the reaction center is less electropositive than the O_2/H_2O redox couple. Thus, the reduction of coenzymes requires electron donors more reduced than water (reduced sulfur compounds,

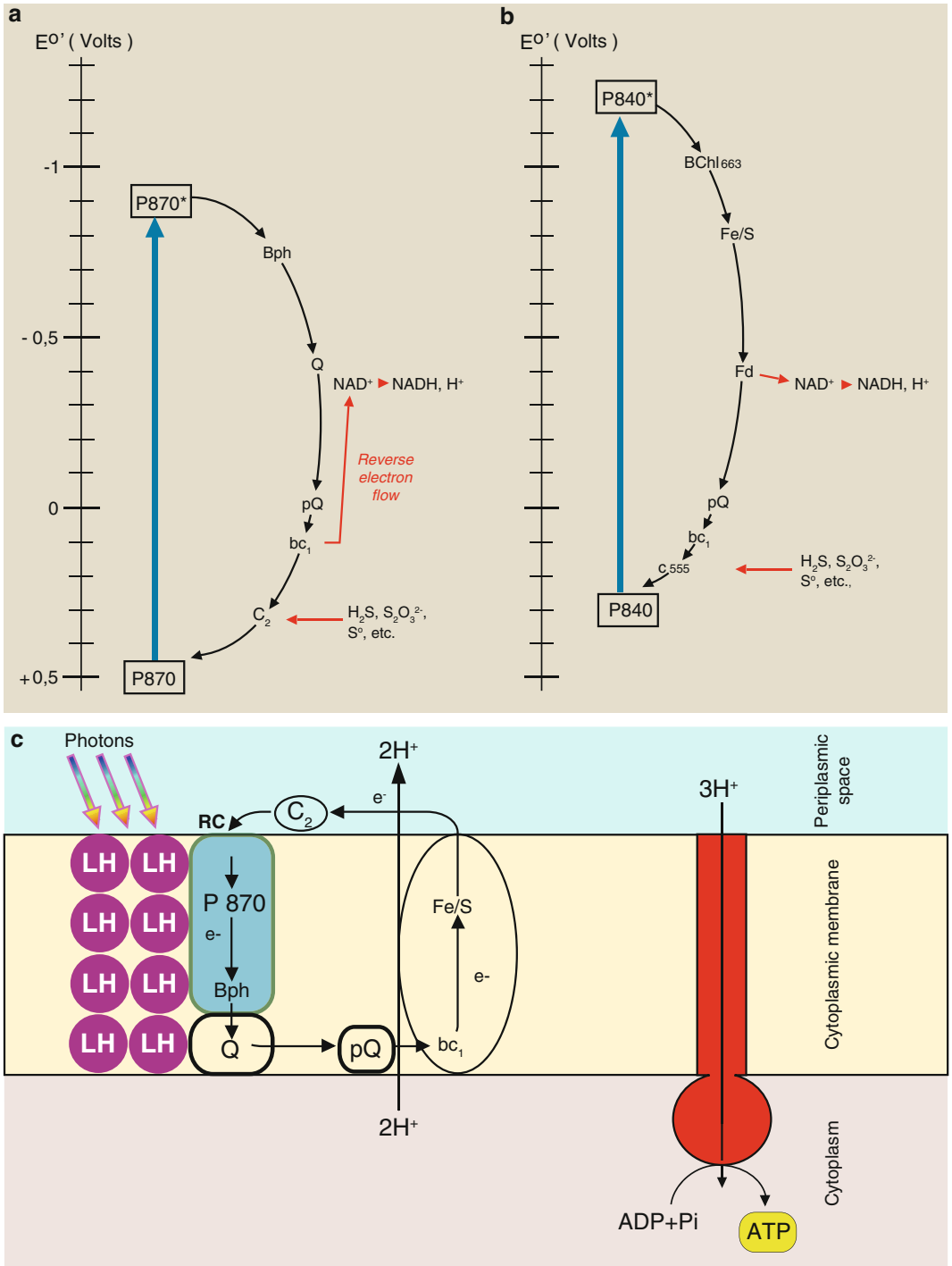

Fig. 3.29 Photosynthetic system of anoxygenic phototrophic bacteria. (**a**) Purple phototrophic bacteria. (**b**) Green sulfur phototrophic bacteria. (**c**) Membrane localization of the photosynthetic apparatus of purple bacteria, proton transfer, and photophosphorylation. *P840 and P870* bacteriochlorophylls of reaction centers (RC), *P870* and P840** excited bacteriochlorophylls, *Bph* bacteriopheophytin, *BChl* $_{663}$ amended bacteriochlorophyll a, *Q* quinone, *pQ* quinone pool, *Fe/S* iron–sulfur protein, *Fd* ferredoxin, *bc1* complex of cytochromes b and c1, *c2* cytochrome c2, and *c555* cytochrome c555. *LH* right harvesting pigments. *RC* reaction center (Drawing: M.-J. Bodiou)

H_2, organic compounds). Anoxygenic phototrophic bacteria so have an anaerobic photosynthetic activity.

Anoxygenic phototrophic activity (photosystem of type II) was demonstrated in planktonic aerobic heterotrophic bacteria or aerobic anoxygenic phototrophs (*Erythrobacter*, *Roseobacter*, *Erythromicrobium*, *Roseococcus*, *Porphyrobacter*, etc.). However, photophosphorylations only provide a supplementary supply of energy, unable to ensure by itself the growth of these microorganisms (Yurkov and Beatty 1998; Fuchs et al. 2007).

The BChl *a* (P870 or P840) excited by light transfers its electrons to an electron acceptor at low potential (Bph or BChl 663). The electrons are then transferred to a series of electron carriers. During this transfer, the electron energy level drops. Finally, these low-energy electrons return to the reaction centers and reduce again the Bchl. During the cycle, protons are transferred to the outside of the cytoplasmic membrane. H^+ gradient is thus created; it is at the origin of a proton-motive force that generates ATP by photophosphorylation. The reduction of NAD^+ is carried by electrons coming out of cyclical flow, thus causing a deficit of electrons. This is compensated by a contribution from inorganic external donors (reduced sulfur compounds, H_2) or organic (succinate, malate) via the cytochromes C555 or C2 in the case of sulfur compounds and plastoquinone (PQ) in that of the succinate. The electrons return to reaction centers where they make up the deficit of BChl which is consecutive to its excitement. In the case of phototrophic purple bacteria, the electrons which reduce coenzymes pass via the complex cytochrome b/c1 (Fig. 3.29a). The positive redox potential of this complex makes impossible the direct reduction of NAD^+ whose redox potential is very negative (−0.32 V). A reverse flow of electrons that consumes energy is needed to allow the reduction of coenzymes. In phototrophic green bacteria, the redox potential of ferredoxin is sufficiently electronegative (−0.42 V) to directly reduce coenzymes (Fig. 3.29b).

3.3.4.4 Use of Light Energy by the Archaea

Some extreme halophilic archaea, *Halobacterium salinarum* in particular, can use light as an energy source when dioxygen levels in their natural environment are too low. The membrane of archaea is largely invaded by dark red color that characterizes the purple membrane containing a colored protein, bacteriorhodopsin, near the rhodopsin of the retina of eyes. Bacteriorhodopsin has a carotenoid as prosthetic group, the all-trans-retinal bound by a **Schiff base*** which serves as photoreceptor. When the all-trans-retinal is activated by a photon, it isomerizes into 11-cis-retinal causing a change in the pattern of the protein, and the Schiff base loses a proton (Fig. 3.30) which is excreted to the outside. Then, the cis-retinal returns to its stable form of trans-retinal and

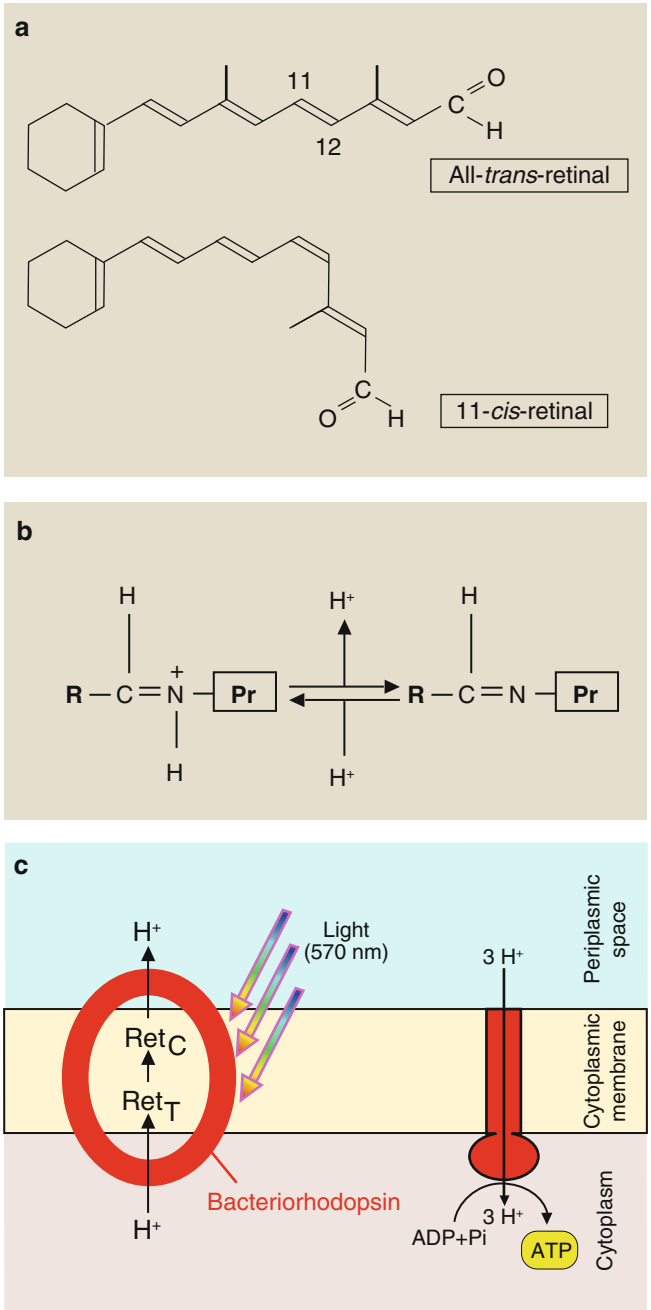

Fig. 3.30 Scheme of proton transfer by bacteriorhodopsin. (**a**) The two retinal isomers. (**b**) The mechanism of proton transfer by bacteriorhodopsin. Retinal (R) and protein (Pr) linked by a Schiff base. (**c**) Scheme of proton transfer and formation of proton-motive force in the purple membrane of halobacteria. *RetC* cis-retinal and *RetT* trans-retinal (Drawing: M.-J. Bodiou)

the Schiff base recovers a cytoplasmic proton. A new isomerization process can take place. Thus, bacteriorhodopsin acts as proton pump transferring protons from the inside to the outside of the cell under the influence of light. It establishes a proton gradient which results in a proton-motive force, an

energy source for cells. Proteorhodopsin, a new rhodopsin similar to bacteriorhodopsin of the archaea, is widely distributed in marine bacteria and functions as a proton pump light dependent (Walter et al. 2007).

3.4 Production of Cellular Material and Biosyntheses

An important part of the energy produced by the cells is used to synthesize their cellular constituents (biosynthesis); this is anabolism. From inorganic and/or simple organic compounds, microorganisms produce more and more complex molecules at the origin of all structural and functional components of cells (Fig. 3.31).

The autotrophic microorganisms synthesize carbohydrates, lipids, proteins, nucleotides, and other constituents from inorganic molecules and ions: CO_2, ammonium, sulfate, phosphate, etc. The heterotrophic microorganisms depend on the organic molecules produced by the autotrophs. They are unable to synthesize their organic molecules from CO_2 and even sometimes prefer to use organic nitrogen of the amino acids. Several require growth factors, essential organic compounds they are unable to synthesize: vitamins, essential amino acids, purines, etc.

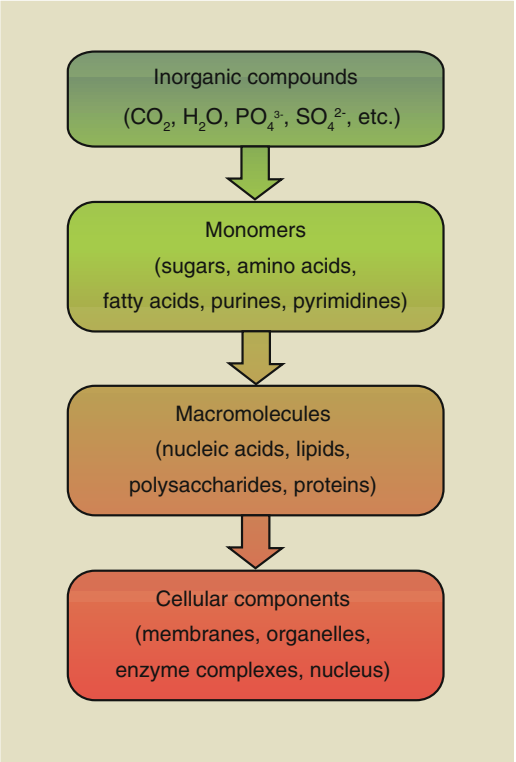

Fig. 3.31 Main steps of the synthesis of cellular constituents (Drawing: M.-J. Bodiou)

3.4.1 The Autotrophic Microorganisms: CO_2 Assimilation

Several metabolic pathways are known in microorganisms for assimilation (or fixation) of CO_2:

1. The pathway of ribulose 1,5-diphosphate or Calvin cycle
2. The reverse cycle of tricarboxylic acids
3. The reductive pathway of acetyl-CoA
4. The cycle of 3-hydroxypropionate
5. The pathway of C4

3.4.1.1 The Pathway of Ribulose 1,5-Diphosphate

This pathway or Calvin cycle (Calvin–Benson cycle, Calvin–Benson–Bassham cycle, or C3 cycle) is one of the most important biosynthetic processes of the biosphere. It is used to fix CO_2 by many autotrophic microorganisms (photosynthetic microeukaryotes, cyanobacteria, anoxygenic phototrophic purple bacteria, chemolithotrophic bacteria) and by plants.

The key enzyme of the Calvin cycle is ribulose 1,5-bisphosphate carboxylase or RuBisCo. It catalyzes the carboxylation of ribulose 1,5-diphosphate and thus allows fixing a CO_2 molecule in an organic form. The Calvin cycle can be divided into three phases (Fig. 3.32). Phase I corresponds to the phase of the fixation of three CO_2 molecules by reaction with three molecules of ribulose 1,5-diphosphate to form six molecules of 3-phosphoglycerate. During phase II or phase of reduction, the six molecules of 3-phosphoglycerate are reduced to 3-phosphoglyceraldehyde (PGA), one molecule being reserved for biosynthesis and the other five used to regenerate three molecules of ribulose 1,5-diphosphate in a series of complex biochemical reactions (phase III). Thus, one molecule of 3-phosphoglyceraldehyde (C3) is synthesized by the Calvin cycle from three molecules of CO_2:

$$3\,CO_2 + 9ATP + 6\,NADH, H^+ (\text{or } NADPH, H^+) \rightarrow 1\,PGA + 9\,ADP + 8Pi + 6\,NAD^+ (\text{or } NADP^+)$$

To synthesize one molecule of PGA, nine ATP and six NADPH, H^+ are needed; CO_2 fixation is a process that requires considerable energy and reducing power. If the light is an inexhaustible source of energy available to phototrophic microorganisms, it is not the same for chemolithotrophic microorganisms where oxidation of the energy source usually generates little free energy. For example, in the case of *Nitrobacter* (nitrite-oxidizing bacteria), it is generally accepted that the oxidation of one ion-gram of NO_2^- allows the translocation of two protons that generate 0.6 mole of ATP. Moreover, the reduction of coenzymes involves a reverse electron flow that consumes six ATP by

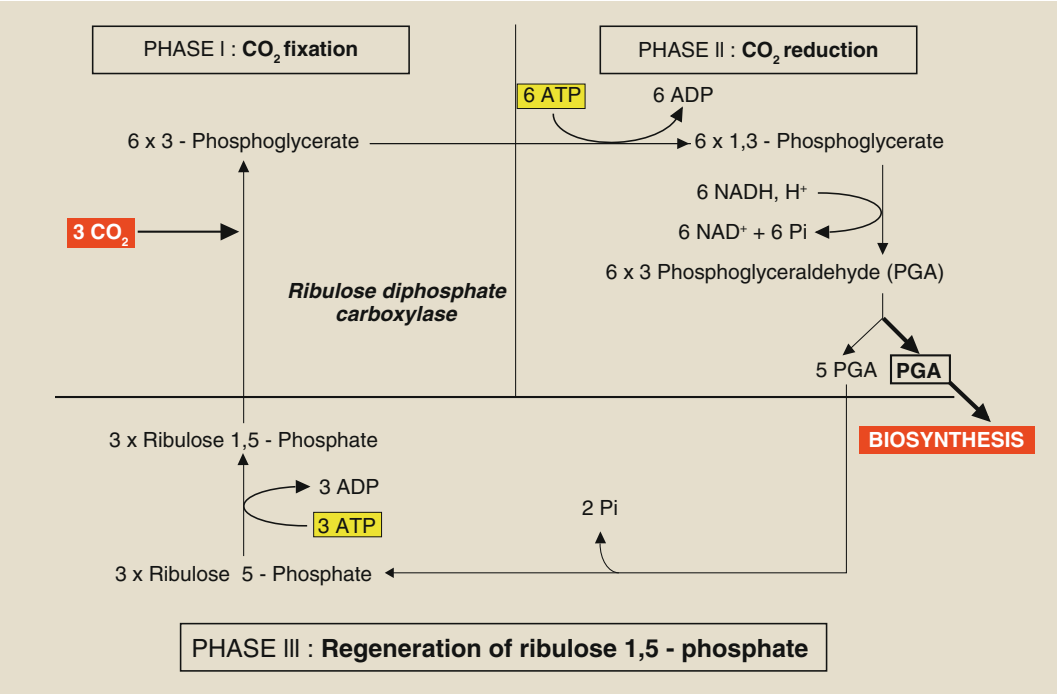

Fig. 3.32 The Calvin cycle (Drawing: M.-J. Bodiou)

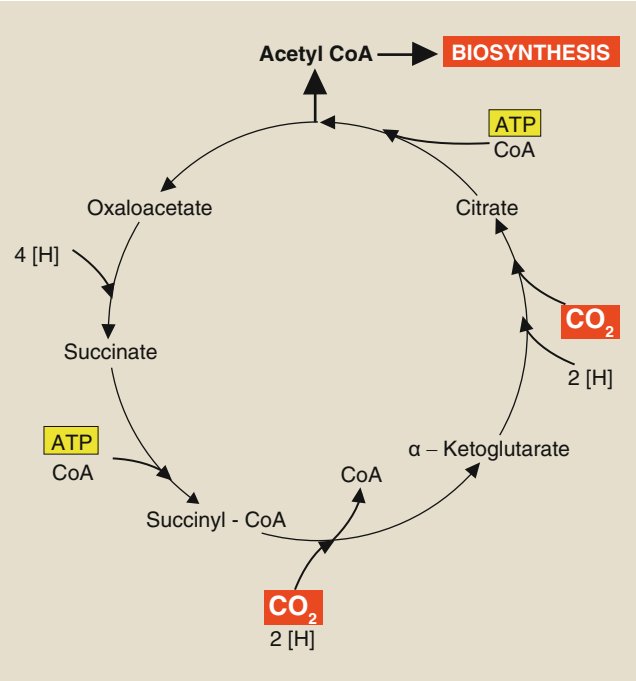

Fig. 3.33 The reverse tricarboxylic acid cycle (Drawing: M.-J. Bodiou)

reduced NAD^+. Thus, the reduction of three moles of CO_2 to form one mole of PGA requires 45 ATP or 75 ion-grams of NO_2^-. In these circumstances, it is clearly conceivable that these microorganisms oxidize a large amount of substrate and growth based on the biosynthesis is slow.

The Calvin cycle takes place in the stroma of chloroplasts in photosynthetic eukaryotes where RuBisCo is soluble, or is located in the pyrenoid in algae having this structure. In some prokaryotes, photosynthetic or non-photosynthetic (cyanobacteria, nitrifying bacteria, and aerobic sulfur-oxidizing bacteria), the Calvin cycle occurs in the cytoplasm where the RuBisCo is condensed as crystalline cytoplasmic inclusions, the carboxysomes. Studies have shown activity of RuBisCo in archaea (*Thermococcus*, *Archaeoglobus*, *Pyrococcus*, methanogens), although no Calvin cycle has been clearly identified (Mueller-Cajar and Badger 2007).

3.4.1.2 The Reverse Tricarboxylic Acid Cycle

Phototrophic green bacteria (*Chlorobium*), hydrogenotrophic bacteria (*Hydrogenobacter*), sulfate-reducing bacteria (*Desulfobacter*), and sulfur-reducing archaea (*Thermoproteus*, *Pyrobaculum*) fix CO_2 during the reverse cycle of tricarboxylic acids (Fig. 3.33). The steps from ketoglutarate to succinate and citrate to oxaloacetate are nonreversible steps of the normal cycle of Krebs. They are catalyzed by new enzymes and require energy. Each running cycle uses two ATP and eight reducing equivalents to reduce two CO_2 to obtain one molecule of acetyl-CoA reserved for cellular synthesis.

3.4.1.3 The Acetyl-CoA Reductive Pathway

Acetyl-CoA reductive pathway or Wood–Ljungdahl pathway described in acetogenic bacteria (*cf.* Sect. 3.3.2) allows

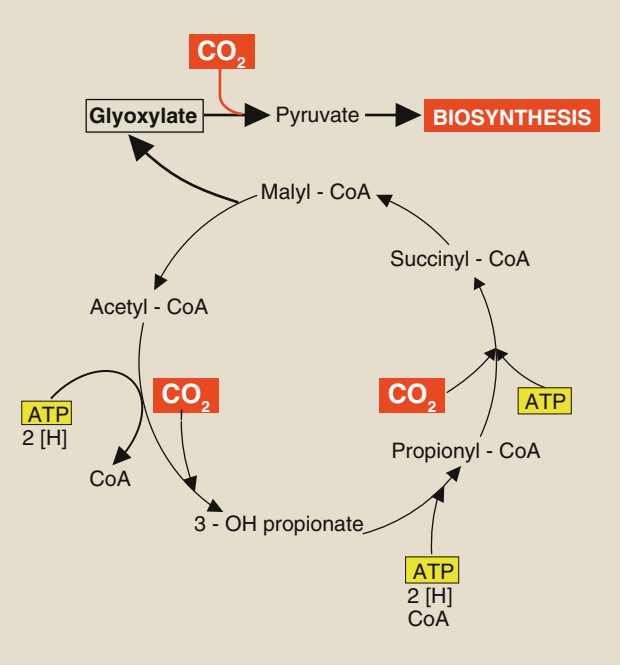

Fig. 3.34 The 3-hydroxypropionate cycle (Drawing: M.-J. Bodiou)

CO_2 fixation by most acetogenic bacteria, few sulfate-reducing bacteria (*Desulfobacterium*), or archaea (*Archaeoglobus*, methanogens).

3.4.1.4 The 3-Hydroxypropionate Cycle

3-Hydroxypropionate cycle is the way of CO_2 fixation in *Chloroflexus* (filamentous phototrophic green nonsulfur bacteria) and in some autotrophic archaea (*Sulfolobus*, *Acidianus*, *Metallosphaera*). During the cycle (Fig. 3.34), three ATP and four reducing equivalents are used to fix a first CO_2 in a C2 unit and a second CO_2 in a C3 unit to form a C4 compound (malyl-CoA). The enzymatic cleavage of malyl-CoA releases glyoxylate subsequently used in biosynthetic pathways and regenerates acetyl-CoA.

Zarzycki et al. (2009) proposed for *Chloroflexus aurantiacus*, a mechanism of CO_2 fixation involving the combination of two cycles: a previous cycle produces glyoxylate that is consumed by a second cycle which releases pyruvate for biosynthesis.

3.4.1.5 The C4 Pathway

Abundant in the marine environment, inorganic carbon is mainly in the form of bicarbonate. Normally, to provide a good functioning of the Calvin cycle or C3 pathway, the intracellular concentration of CO_2 must be high. In algae, carbonic anhydrase which interconverts CO_2 and bicarbonate is the main component of the mechanism of intracellular concentration of inorganic carbon that feeds the Calvin cycle:

$$HCO_3^- + H^+ \leftrightarrow CO_2 + H_2O$$

Another pathway optimizes the absorption and storage of CO_2 in the cells. This way or C4 pathway, known in terrestrial plants from hot and dry climate, has been described for diatoms which represent a significant fraction of the oceanic phytoplankton. The source of inorganic carbon is bicarbonate ion. This ion reacts with phosphoenolpyruvate to form a C4 compound, oxaloacetate, under the action of carboxylase. Oxaloacetate produces malate which is a form of CO_2 storage. Decarboxylation of malate produced pyruvate used in the biosynthesis and CO_2 which supplies the Calvin cycle.

In diatoms, the C4 pathway (Fig. 3.35) occurs in the cytoplasm and the Calvin cycle in the chloroplast. Diatoms in their natural environment are subject to significant fluctuations of light intensities depending on their position in the water column. At low light intensities, the C4 pathway allows storage of CO_2 in the form of malate. This CO_2 is released and used as a supplement during periods of high demand for high light intensities.

3.4.1.6 Other Ways of CO_2 Fixation

Two ways of CO_2 fixation, the hydroxypropionate–hydroxybutyrate and dicarboxylate–hydroxybutyrate cycles, have been described in archaea (Berg et al. 2010).

3.4.2 C1 Compound Assimilation and Related Compounds

The reduced C1 compounds (methane, methanol, formaldehyde, chloromethane), methylamines and methylated sulfur compounds, are assimilated by microorganisms, especially aerobic and anaerobic prokaryotes, microorganisms known as **methylotrophs***. The assimilation of these compounds is less costly in energy than CO_2 fixation because it requires less ATP and less reduced coenzymes.

3.4.2.1 Aerobic Methylotrophic Microorganisms

Some bacteria are obligate aerobic methylotrophs (*Methylobacter*, *Methylocystis*, *Methylobacillus*, etc.), and others may be facultative methylotrophs also able to use substrates containing more than one carbon atom (*Methylobacterium*, *Methylosulfonomonas*, *Paracoccus*, *Bacillus*, etc.). Among the obligate methylotrophs, only methanotrophic bacteria are capable of assimilating methane (*Methylobacter*, *Methylocystis*).

The C1 compounds are assimilated as formaldehyde (Fig. 3.36). The fixation of formaldehyde involves the

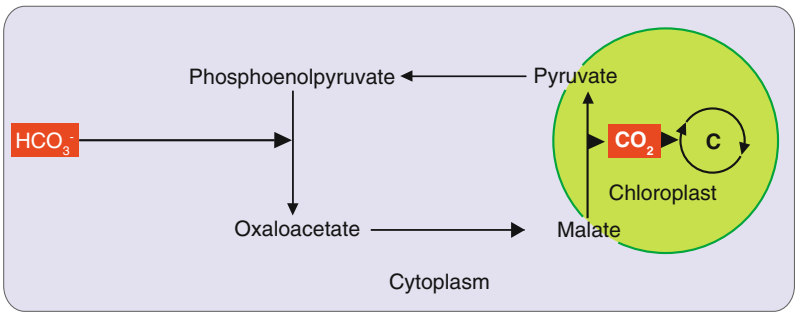

Fig. 3.35 The C4 pathway of diatoms (Modified and redrawn from Riebesell 2000). *c* Calvin cycle (Drawing: M.-J. Bodiou)

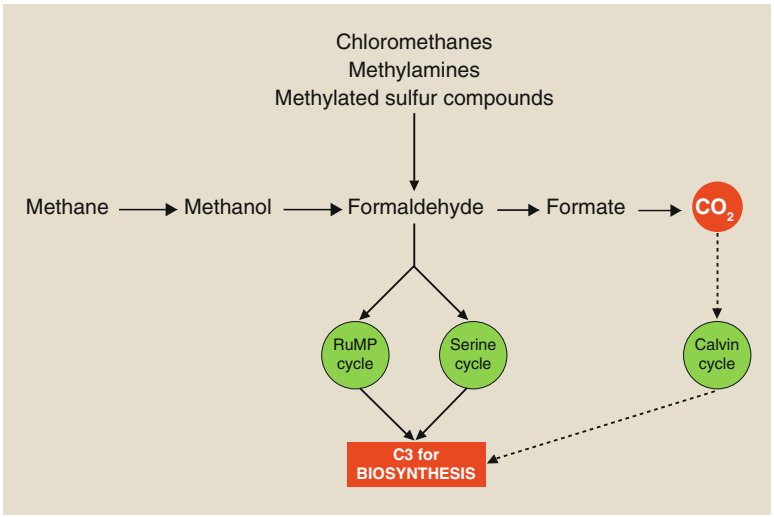

Fig. 3.36 Assimilation of C1 compounds (Modified and redrawn from Lidstrom 1991. Drawing: M.-J. Bodiou)

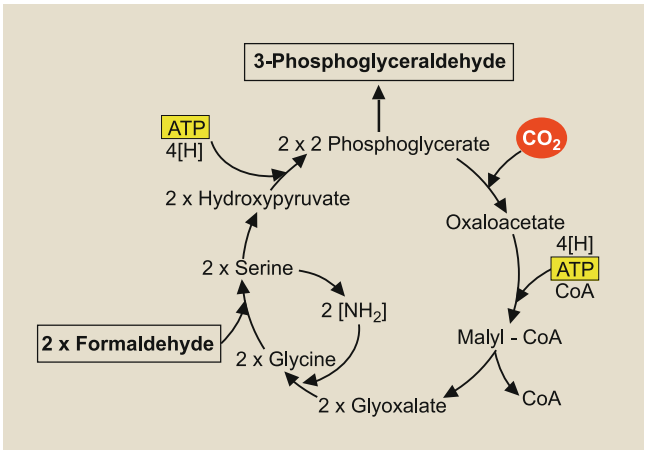

Fig. 3.37 The cycle of serine (Drawing: M.-J. Bodiou)

condensation with a compound containing more than one carbon atom and the production of a C3 molecule that feeds the biosynthesis. Two cycles are responsible for this fixation, the serine cycle (Fig. 3.37) and the ribulose monophosphate cycle (Fig. 3.38).

The formaldehyde is condensed with glycine (Fig. 3.37) or ribulose 5-phosphate (Fig. 3.38) to form glycerate 3-phosphate or dihydroxyacetone 3-phosphate, respectively. Bacteria known as pseudomethylotrophs can oxidize C1 to CO_2 which is then used by the Calvin cycle. Formaldehyde is also assimilated by eukaryotic microorganisms (yeasts) by condensation with xylulose 5-phosphate which produces glyceraldehyde 3-phosphate and dihydroxyacetone 3-phosphate.

3.4.2.2 Anaerobic Methylotrophic Microorganisms

Formation of acetyl-CoA described among acetogenic bacteria (*cf.* Sect. 3.3.2) is the usual way of fixing reduced C1 compounds among anaerobic microorganisms: methanogens and acetogens.

3.4.3 Heterotrophic Microorganisms

The heterotrophic microorganisms require organic compounds for growth. These compounds restore energy in various forms (proton-motive force, ATP, reduced coenzymes) and provide the carbon skeletons that are used in the biosynthesis.

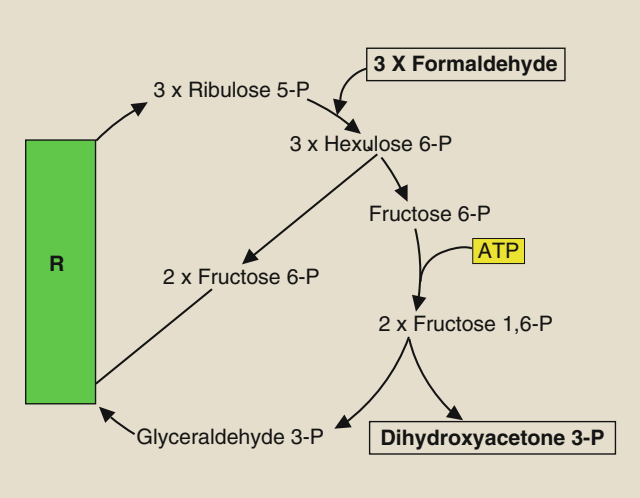

Fig. 3.38 The cycle of ribulose monophosphate (RUMP). *R* regeneration phase of the ribulose 5-P; *P* phosphate group (Drawing: M.-J. Bodiou)

In natural environments, the most abundant organic substances available to the microorganisms are macromolecules or biopolymers (polysaccharides, proteins, lipids, nucleic acids, etc.) that cannot penetrate into cells through the cytoplasmic membranes. Most heterotrophic microorganisms must excrete extracellular enzymes or exoenzymes that divide the polymer into small molecules (monomers) more easily transported inside the cells. The exoenzymes are mainly hydrolases that degrade polysaccharides (amylases, cellulases, pectinases, chitinases, xylanases, etc.), proteins (proteases), lipids (lipases), and nucleic acids (nucleases).

Some macromolecules are not degraded by hydrolases. This is, for example, the case of lignins that require the presence of polyphenoloxidases.

Unlike these enzymes that are released in the environment as true exoenzymes, certain enzymes involved in degradation of polymers can be fixed on the cell surfaces. Cellulolytic bacteria such as *Clostridium* and *Bacteroides* synthesize large protrusions attached on their surface, called cellulosomes. The cellulosomes are composed of multienzyme complexes containing endocellulases (enzymes hydrolyzing the bonds within the polysaccharide chain), xylanases, and other degradative enzymes. The attachment of cellulosomes to cellulose fibers provides intimate contact that allows the hydrolysis of the polymer.

Protozoa incorporate organic particles in their cells (organic debris, bacteria) which they feed by endocytosis. Monomers (amino acids, sugars) can also enter by endocytosis, by diffusion, or with the aid of carriers.

The hydrolysis of polysaccharides essentially provides hexoses. Lipases hydrolyze lipids into glycerol and fatty acids that penetrate the cells. During the β-oxidation, the fatty acids are cut into acetyl-CoA molecules. Proteases hydrolyze proteins into amino acids which are mostly deaminated inside cells into their corresponding keto acids. Nucleases hydrolyze nucleotides in nucleic acids that are absorbed by the cells after dephosphorylation and then degraded. All of these simple molecules resulting from enzymatic activities become the metabolic precursors of biosyntheses. They enter into the **central metabolic pathways*** to produce the monomers required for the synthesis of cellular macromolecules.

3.4.3.1 Assimilation of C2 Compounds

Some heterotrophic microorganisms can grow on C2 compounds as sole carbon source. To assimilate acetate, anaerobic bacteria activate the molecule in the form of acetyl-CoA, and then a following carboxylation produces pyruvate under reducing conditions. Aerobic microorganisms assimilate acetyl-CoA through the glyoxylate cycle (Fig. 3.40); other C2 compounds, glycine, glycolate, and oxalate, are first converted to glyoxylate and finally glycerate. Other metabolic pathways have been described for acetate assimilation such as citramalate cycle or acetoacetyl-CoA pathway.

3.4.3.2 Central Metabolic Pathways and Formation of the Carbon Skeleton of the Main Monomers

The central metabolic pathways (Fig. 3.39) are common to the vast majority of eukaryotic and prokaryotic microorganisms. Four major pathways are involved:

1. The glycolysis
2. The neoglucogenesis
3. The tricarboxylic acid cycle (TCA) or Krebs cycle
4. The pentose phosphate cycle

The carbon skeletons of the main monomers originate from metabolic intermediates of these pathways. The biosyntheses of purines and pyrimidines are complex reactions involving several sources of carbon and nitrogen.

Some steps of neoglucogenesis are nonreversible steps of glycolysis. The conversion of pyruvate to phosphoenolpyruvate occurs in two steps: a step of carboxylation to oxaloacetate and a decarboxylation step of oxaloacetate to phosphoenolpyruvate under the respective actions of a carboxylase and a carboxykinase with energy consumption (Fig. 3.39). The direct phosphorylation of pyruvate to phosphoenolpyruvate was described in Gram-negative bacteria. This reaction is catalyzed by phosphoenolpyruvate synthase and requires two energy-rich phosphate bonds. Two other steps of glycolysis are not reversible, dephosphorylation of fructose 1,6-P and of glucose-6-P which depend on the action of two specific phosphatases.

Biosynthesis pathways are less known in *Archaea*. The reverse path of glycolysis works even if the degradation of glucose does not always pass by this path. A complete cycle of oxidative citric acid, involving the same enzymes of bacteria, operates in aerobic archaea (archaeal halophiles,

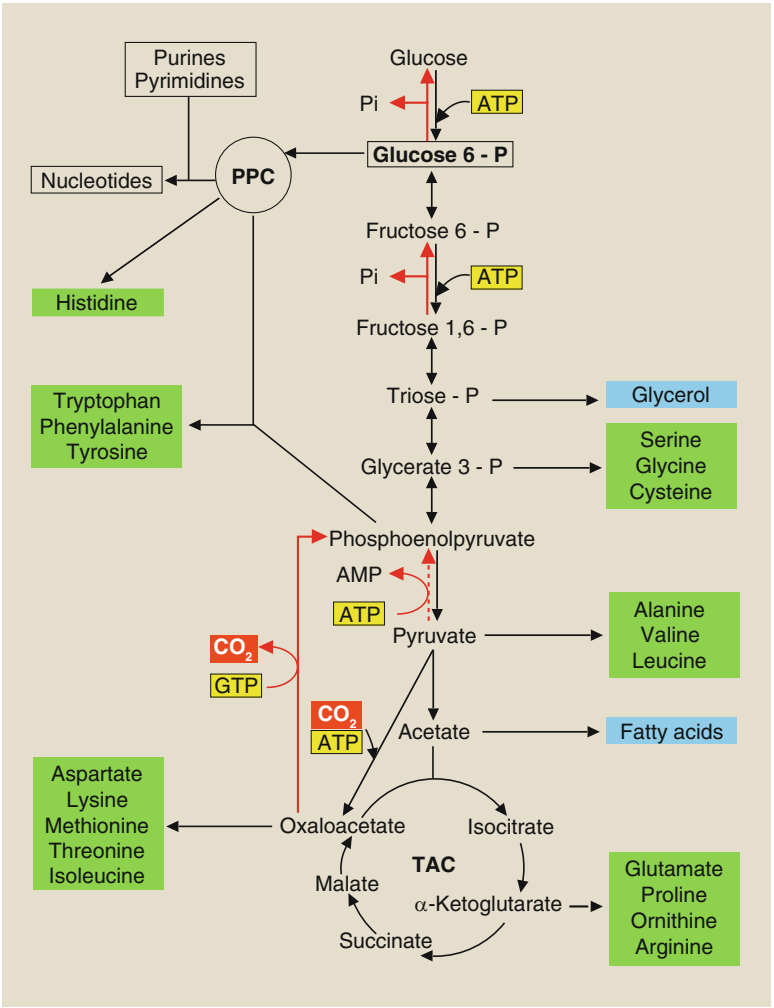

Fig. 3.39 The central metabolic pathways, formation of carbon skeletons of the main monomers. *Gluconeogenesis* nonreversible steps of glycolysis are in *red*, *Pi* inorganic phosphate, *PPC* pentose phosphate cycle, *TAC* tricarboxylic acid cycle (Krebs cycle), *P* phosphate group, and *Frames* origin of the carbon skeletons of the monomers (Drawing: M.-J. Bodiou)

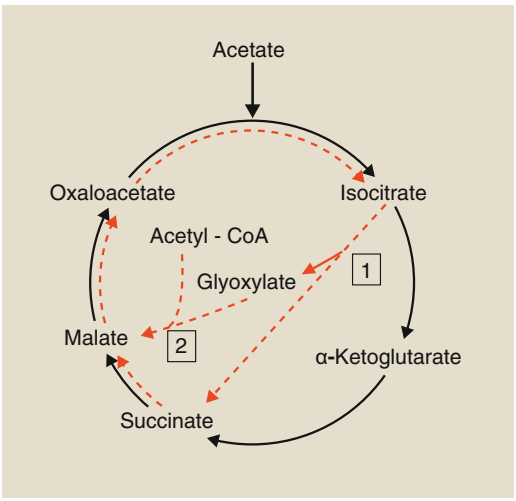

Fig. 3.40 Scheme of the glyoxylate cycle (in *red* in the Krebs cycle). *Black arrows*, normal citric acid; *1*, isocitrate lyase; and *2*, malate synthase (Drawing: M.-J. Bodiou)

Thermoplasma, *Sulfolobus*), while an incomplete cycle was often described in the anaerobic archaea (*Pyrococcus*, *Methanosarcina*, *Archaeoglobus*) (Danson et al. 2007). In some archaea, the whole of genes encoding enzymes of the cycle has been identified (*Halobacterium*, *Thermoplasma*, *Picrophilus*). In others, the cycle can run in the direction of reduction (reverse cycle) for CO_2 fixation during autotrophic growth (Hu and Holden 2006).

The oxaloacetate is required for operating the Krebs cycle. The removal of this compound for biosyntheses (neoglucogenesis, amino acid synthesis) must be compensated for the cycle to continue to operate. Reactions providing the compound or precursors are **anaplerotic sequences***. These reactions catalyzed by a carboxylase (3.6) and a carboxykinase (3.7) produce oxaloacetate:

$$\text{Pyruvate} + CO_2 + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP} + \text{Pi} \quad (3.6)$$

$$\text{Phosphoenolpyruvate} + CO_2 + \text{GDP} \leftrightarrow \text{oxaloacetate} + \text{GTP} \quad (3.7)$$

Table 3.16 Main monomers required for the biosynthesis of macromolecules

Monomers	Polymers
Glycerol and fatty acids	Lipids
Glucose 6-phosphate	Polysaccharides
Amino acids	Proteins
Pentose phosphates and purines or pyrimidines	Nucleic acids

Table 3.17 Properties of different nitrate reductases

	Assimilatory nitrate reductase (Nas)	Dissimilatory nitrate reductase (Nar)	Periplasmic nitrate reductase (Nap)
Location	Cytoplasm	Membrane	Periplasm
Inhibition by O_2	No	Yes	No
Inhibition by NH_3	Yes	No	No
Enzymatic induction	No[a]	Yes	No[a]
Function	NO_3^- assimilation	Anaerobic respiration of nitrate	Regulation of redox potential[b]

[a]Assimilative and periplasmic nitrate reductases are constitutive
[b]Nap removes excess reducing power by producing toxic NO_2^- for eventual competitors or providing NO_2^- to nitrite reductase, which allows microorganisms to adapt to rapid changes in oxygenation

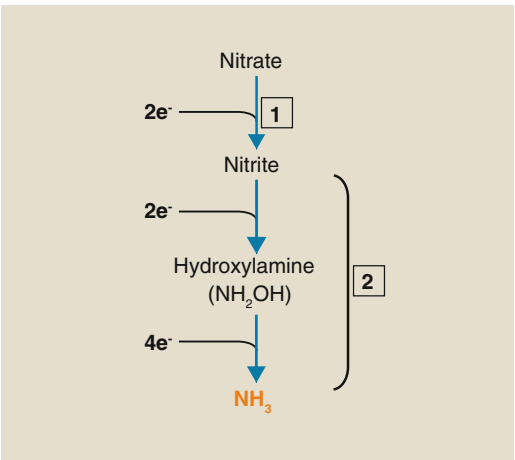

Fig. 3.41 The assimilatory reduction of nitrate. *1* nitrate reductase Nas; *2* nitrite reductase (Drawing: M.-J. Bodiou)

Another source of oxaloacetate is the formation of glyoxylate from isocitrate, which shortens the Krebs cycle avoiding the step of ketoglutarate (Fig. 3.40). The shorter Krebs cycle is called glyoxylate cycle.

The main monomers synthesized from different carbon skeletons are used to build macromolecules (Table 3.16).

3.4.4 Assimilation of Nitrogen, Sulfur, and Essential Elements

3.4.4.1 Assimilation of Nitrogen Compounds

The most abundant nitrogen sources for microorganisms are ammonia nitrogen, nitrate, and dinitrogen. Only ammonia nitrogen is directly assimilated by the microorganisms. Nitrate and dinitrogen must be reduced to ammonia to be assimilated. Protozoa are dependent to organic nitrogen (*cf.* Sect. 14.3.2).

Assimilatory Reduction of Nitrate

Nitrate is reduced to nitrite by assimilatory nitrate reductase (Nas) whose properties are different from dissimilative reductases previously described (Table 3.17) and used in energy metabolism. Nitrite is then reduced to ammonia in several steps by a nitrite assimilatory reductase (Fig. 3.41). The reduction of nitrate to ammonium nitrogen is coupled to a high consumption of ATP and reducing power (reduced coenzymes):

$$NO_3^- + 8e^- + 9H^+ \rightarrow NH_3 + 3H_2O$$

Microorganisms unable to reduce nitrate must find the ammonia nitrogen in their environment.

Dinitrogen Fixation

Many aerobic and anaerobic microorganisms can assimilate dinitrogen (Table 3.18). This assimilation or dinitrogen fixation is under the control of an enzyme complex called nitrogenase complex. Nitrogenase is very sensitive to dioxygen. Among aerobic heterotrophs, nitrogen fixation occurs when the respiration equals or exceeds the rate of dioxygen diffusion into the cells. In some filamentous cyanobacteria, nitrogenase is localized in specialized cells (heterocysts) devoid of photosystem II which produces dioxygen.

Nitrogenase is a metalloprotein complex. Some nitrogenases contain vanadium or iron, but the most common nitrogenases contain molybdenum. The functional enzyme is composed of two types of soluble proteins: one is molybdenum iron protein (MoFe) or dinitrogenase, and the other is known as protein iron (Fe) or dinitrogenase reductase. In a first step, a flavodoxin or ferredoxin is reduced (Fig. 3.42). Then the dinitrogenase reductase accepts electrons and transmits them to the dinitrogenase that reduces dinitrogen into ammonia. ATP needs are important to reduce the triple bond linking the two nitrogen atoms:

$$N_2 + 8\,e^- + 8\,H^+ + 16\,ATP \rightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,Pi$$

If microorganisms are provided with another source of assimilable nitrogen, they do not fix dinitrogen. Ammonia nitrogen also represses the synthesis of nitrogenase complex.

Table 3.18 Examples of microorganisms fixing dinitrogen under nonsymbiotic conditions

Chemotrophic microorganisms	Phototrophic microorganisms
Aerobic microorganisms	*Aerobic microorganisms*
Azotobacter, *Azomonas, Klebsiella*, *Azospirillum, Gluconacetobacter, Bacillus, Thiobacillus*, *Alcaligenes*, methylotrophic bacteria	*Cyanobacteria*
Anaerobic microorganisms	*Anaerobic microorganisms*
Bacteria: *Clostridium*, sulfate-reducing bacteria	Anoxygenic phototrophic bacteria
Archaea: methanogens	

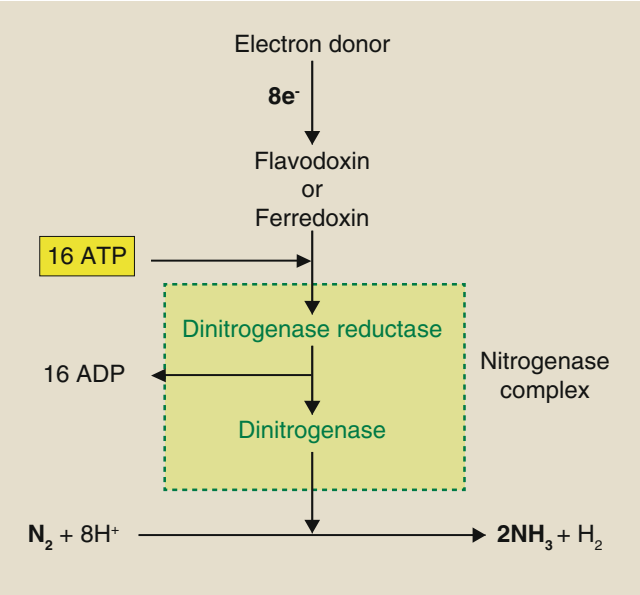

Fig. 3.42 Steps of dinitrogen fixation (Drawing: M.-J. Bodiou)

The bacteria mentioned above that fix dinitrogen without any symbiotic association are called free nitrogen-fixing bacteria. Bacteria known as symbiotic nitrogen-fixing bacteria fix dinitrogen only in association with plants.

Rhizobium and other genera (*Bradyrhizobium*, *Azorhizobium*), belonging to alphaproteobacteria, infect the roots of legumes, and these cause the formation of nodules. In the nodules, the cells of *Rhizobium* are isolated from the plant material by the surrounding membranes called sequestration membranes. Inside the sequestration membrane, the bacteria continue to multiply at the expense of plant metabolites and then differentiate into bacteroids fixing dinitrogen. In this form, the multiplication of bacteria stops. The leghemoglobin that is present in the nodules is synthesized in part by the plant (globin) and partly by the bacteroids (heme). This molecule regulates the supply of dioxygen to the microorganisms and thereby protects nitrogenase and allows proper operation. Bacteroids fix dinitrogen and release of ammonia nitrogen assimilated by the plant that provides organic energy sources for microorganisms.

The symbiotic nitrogen-fixing actinomycetes of the genus *Frankia* establish associations with different plants. They form root nodules with woody plants such as alders and various other plants belonging to the families Rhamnaceae, Myricaceae, Rosaceae, etc. In general, dinitrogen fixation by free *Frankia*, but also often by symbiotic *Frankia*, is correlated with the presence of vesicles containing nitrogenase. Thick envelopes of these structures limit diffusion of dioxygen and create favorable conditions for enzyme activity.

The presence of free-fixing bacteria associated with plants has been described. Nodules of *Psychotria* leaves (plant of Rubiaceae family) contain *Klebsiella*. Fixing cyanobacteria (*Anabaena*) are associated with aquatic ferns (*Azolla*). Some bacteria of the genus *Gluconacetobacter* are endophytes of tissues of sugarcane; others multiply in the rhizosphere of coffee plants. *Azotobacter* grows in the rhizosphere of corn. It is not certain that in these cases the bacteria that benefit from root exudates contribute to nitrogen nutrition of plants (*cf.* Sect. 11.3).

Incorporation of Ammonium

Microorganisms incorporate ammonia nitrogen in organic compounds by using one of the mechanisms described in the following equations:

$$\alpha\text{-ketoglutarate} + NH_3 + NADPH, H^+ \rightarrow \text{L-glutamate} + H_2O + NADP^+ \quad \textit{glutamate dehydrogenase}$$

$$\text{Pyruvate} + NH_3 + NADPH, H^+ \rightarrow \text{L-alanine} + H_2O + NAD^+ \quad \textit{alanine dehydrogenase}$$

$$\text{Glutamate} + NH_3 + ATP \rightarrow \text{glutamine} + ADP + Pi \quad \textit{glutamine synthetase}$$

$$\alpha\text{-ketoglutarate} + \text{glutamine} + 2\,H^+ + e^- \rightarrow 2\ \text{glutamate} \quad \textit{Glutamine } \alpha\textit{-ketoglutarate aminotransferase}$$

Only a few microorganisms can incorporate ammonia nitrogen in the pyruvate. Glutamate and glutamine are the nitrogen source of the cellular amino acids. Glutamate is the major source of nitrogen.

The other amino acids result from changes to the molecular structure of glutamate to a small number of them (arginine, proline), but essentially from transaminations (transfer reactions of the amino group catalyzed by transaminase) with keto acids (aspartate, arginine, alanine, threonine,

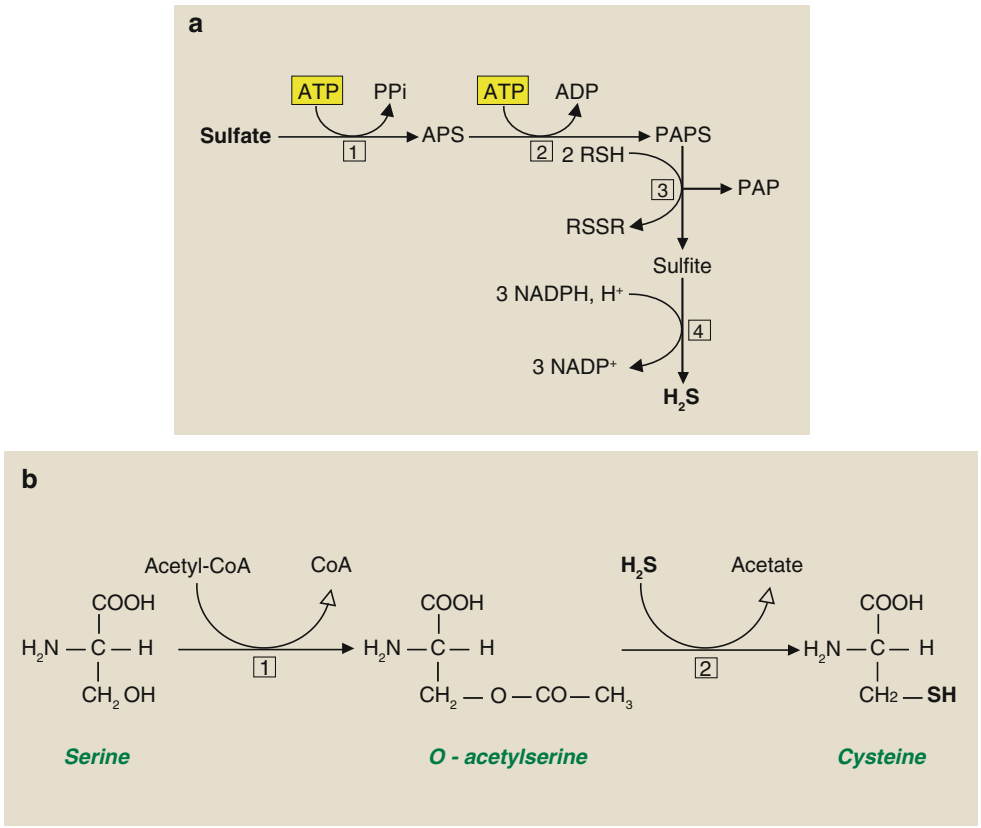

Fig. 3.43 Assimilatory reduction of sulfate (**a**) and incorporation of the sulfide (**b**). (**a**) Steps in the reduction of assimilative sulfate. *1* ATP sulfurylase, *2* phosphokinase, *3* PAPS reductase, *4* sulfite reductase. *RSH* reduced thioredoxin, *RSSR* oxidized form of thioredoxin (RSH regeneration with NADPH, H^+), *APS* adenosine 5′-phosphosulfate, *PAPS* phosphoadenosine 5′-phosphosulfate, and *PAP* phosphoadenosine 5′-phosphate, *PPi* pyrophosphate. (**b**) The incorporation of sulfide to O-acetylserine. *1* Serine transacetylase; *2* O-acetylserine sulfhydrylase (Drawing: M.-J. Bodiou)

isoleucine, methionine, lysine, leucine, valine, serine, glycine, cysteine, phenylalanine, tyrosine). The synthesis of aspartate by transfer of amino group of glutamate to oxaloacetic acid catalyzed by glutamate oxaloacetate transaminase is an example of transamination (*cf.* Sect. 14.3.3, Fig. 14.29):

$$\text{Oxaloacetate} + \text{L-glutamate} \rightarrow \alpha\text{-ketoglutarate} + \text{L-aspartate}$$

Some transamidations (transfer of the amide group of glutamine) are involved in the synthesis of some amino acids (tryptophan, arginine).

The nitrogen of purines and pyrimidines, other important cellular nitrogen compounds, is derived from transamination and transamidation reactions and transfers of carbamyl group (NH_2–CO–) of carbamyl phosphate (NH_2–CO–O–PO_3^{2-}) whose synthesis is shown in the following equation:

$$\text{Glutamine} + CO_2 + 2\ \text{ATP} \rightarrow \text{glutamate} + \text{carbamyl phosphate} + 2\ \text{ADP} + 2\ \text{Pi}$$

Carbamyl phosphate synthase

The synthesis of arginine also implies a carbamyl group. That of histidine is a complex metabolic pathway where the nitrogen has several origins (purine, transamination, and transamidation reactions).

3.4.4.2 Assimilation of Sulfur Compounds

The most abundant form of sulfur in the biosphere is sulfate which is the most oxidized sulfur state (oxidation state + VI) (*cf.* Sect. 14.4.1, Fig. 14.34). Sulfate is the sulfur source used by most microorganisms; animal cells require sulfur in organic form.

In organic matter, sulfur is reduced, mainly in the form of sulfhydryl groups (oxidation state − II). Sulfate represents a too oxidized sulfur source to be included in organic compounds. To be assimilated, it must be reduced to oxidation state − II. The first step in the assimilatory sulfate reduction is the activation in the form adenosine 5′-phosphosulfate or APS (Fig. 3.43). Subsequent phosphorylation leads to the phosphoadenosine 5′-phosphosulfate or PAPS. PAPS is reduced to sulfide in two stages. The sulfide is incorporated into the O-acetylserine to form cysteine. This metabolic pathway has also been described among eukaryotic parasites (*Entamoeba*, *Leishmania*). Cysteine is the main precursor of other sulfur compounds from cells (methionine, coenzyme A, lipoic acid, thiamine, glutathione, iron–sulfur centers).

Microorganisms incapable of assimilatory sulfate reduction must find reduced sulfur compounds in their environment. They are often anaerobic microorganisms that grow in anoxic environments where reduced sulfur compounds are generally abundant.

3.4.4.3 Uptake of Other Inorganic Compounds, Trace Elements, and Essential Factors

Under the action of ATP synthase, phosphate that enters the cell by specific carrier reacts with ADP to give ATP. ATP is the source of phosphate for the synthesis of macromolecules, in particular nucleic acids, phospholipids, and nucleotides.

Microorganisms should find other mineral compounds in their environment such as the general anions and cations which penetrate cells by carrier systems. Potassium, calcium, magnesium, and iron are major elements essential to the cells for several reasons, including enzyme cofactors. Magnesium is present in cell walls, membranes, and phosphoric esters, calcium in coenzymes and bacterial spores in the form of dipicolinate, and iron in metalloproteins (cytochromes, ferredoxins, and iron–sulfur proteins). In oxic environments, extremely insoluble ferric salts are inaccessible to microorganisms. For that reason, microorganisms produce and excrete organic compounds or siderophores that chelate and trap iron (*cf.* Sect. 14.5.2). This chelated iron is absorbed by the cells. However, in anoxic environments, iron present in the form of soluble ferrous iron is readily available to cells.

In general the sodium requirements are low, except in halophilic microorganisms that require higher concentrations of ions Na^+ in their environment and sometimes accumulate in the cytoplasm to compensate osmotic pressure. Diatoms are important needs in silicon, an element essential to the formation of cell walls.

In addition to these compounds, many other elements or trace elements are needed in low concentrations for cellular activities. These are mainly manganese, zinc, molybdenum, selenium, cobalt, nickel, copper, and tungsten. They act as enzyme cofactors or integral part of enzymes.

3.5 Conclusion

Unlike their poorly differentiated structures compared to eukaryotes, prokaryotes have a very diverse metabolism especially in their energy sources (organic or inorganic substrates used through different aerobic and anaerobic respirations, fermentations, or photosynthesis) placing them as key actors with the major role in the transformation of organic or inorganic elements on the planet (*cf.* Chap. 14). Moreover, the metabolic versatility of many prokaryotes gives them a plasticity allowing their adaptation to changing environmental conditions. For example, members of the genus *Shewanella*, facultative anaerobic bacteria that grow in chemolithotrophic conditions with dihydrogen as an energy source or in chemoorganotrophic conditions, exhibit a very large diversity of respiration. In the absence of dioxygen, many of organic or inorganic compounds, including toxic elements and metals, are terminal electron acceptors (trimethylamine oxide, iron III, manganese IV, chromium VI, VI uranium, sulfur, polysulfide, sulfite, thiosulfate, dimethyl sulfoxide, arsenate, succinate, etc.). Similarly, some anoxygenic phototrophic bacteria show great adaptability to environmental conditions. This is the case of representatives of the genus *Thiocapsa* that, under anoxic conditions and in the light, use light radiations as energy source, reduced inorganic compounds of sulfur, dihydrogen, and small organic molecules as electron source, and organic molecules or carbon dioxide for their carbon source. Placed in the dark, under oxic conditions, these microorganisms exhibit an aerobic respiratory mechanism using organic compounds or inorganic sulfur, and under anoxic conditions, they ferment their intracellular reserves of polysaccharides using intracellular sulfur as electron trap. Our knowledge on the adaptability of microorganisms to environmental conditions has increased with the discovery of unexpected metabolic pathways such as aerobic respiration in bacteria long time considered as strictly anaerobic (sulfate-reducing bacteria) and anoxygenic photosynthesis in aerobic chemoorganotrophic heterotrophic bacteria (aerobic anoxygenic phototrophic bacteria).

Prokaryotes are the microorganisms most important in the biodegradation processes, and thus they are used either naturally or artificially in the processes of decontamination and biopurification of anthropized ecosystems (*cf.* Chap. 16). Eukaryotes have developed structures more and more complex with the recovery of both the most energetic prokaryotic metabolisms, both in relation to dioxygen: aerobic respiration and oxygenic photosynthesis.

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