3 General Bacteriology

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The Morphology and Fine Structure of Bacteria

Bacterial cells are between 0.3 and 5 µm in size. They have three basic forms: cocci, straight rods, and curved or spiral rods. The **nucleoid** consists of a very thin, long, circular DNA molecular double strand that is not surrounded by a membrane. Among the nonessential genetic structures are the **plasmids**. The **cytoplasmic membrane** harbors numerous proteins such as permeases, cell wall synthesis enzymes, sensor proteins, secretion system proteins, and, in aerobic bacteria, respiratory chain enzymes. The membrane is surrounded by the **cell wall**, the most important element of which is the supporting murein skeleton. The cell wall of Gram-negative bacteria features a porous outer membrane into the outer surface of which the lipopolysaccharide responsible for the pathogenesis of Gram-negative infections is integrated. The cell wall of Gram-positive bacteria does not possess such an outer membrane. Its murein layer is thicker and contains teichoic acids and wall-associated proteins that contribute to the pathogenic process in Gram-positive infections. Many bacteria have **capsules** made of polysaccharides that protect them from phagocytosis. Attachment **pili** or **fimbriae** facilitate adhesion to host cells. Motile bacteria possess **flagella**. Foreign body infections are caused by bacteria that form a **biofilm** on inert surfaces. Some bacteria produce **spores**, dormant forms that are highly resistant to chemical and physical noxae.

Bacterial Forms

Bacteria differ from other single-cell microorganisms in both their cell structure and size, which varies from 0.3–5 µm. Magnifications of 500–1000×—close to the resolution limits of light microscopy—are required to obtain useful images of bacteria. Another problem is that the structures of objects the size of bacteria offer little visual contrast. Techniques like phase contrast and dark field microscopy, both of which allow for live cell observation, are used to overcome this difficulty. Chemical-staining techniques are also used, but the prepared specimens are dead.

Bacterial Morphology

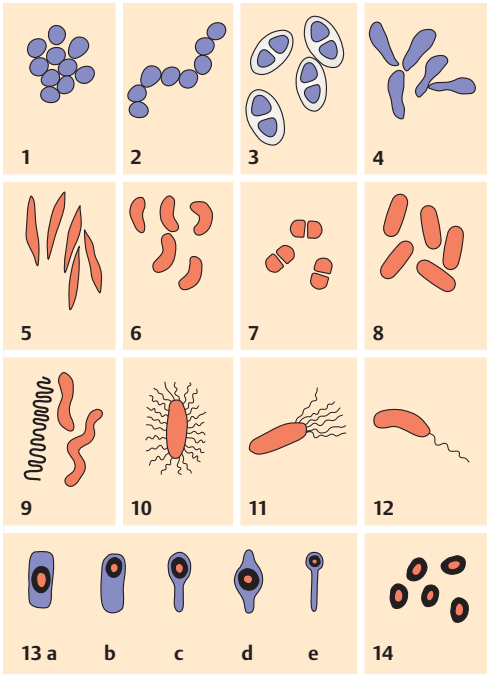

Fig. 3.1

1. Gram-positive cocci in grapelike clusters (staphylococci)
2. Gram-positive cocci in chains (streptococci)
3. Gram-positive cocci with capsules (pneumococci)
4. Gram-positive, clubshaped, pleomorphic rods (corynebacteria)
5. Gram-negative rods with pointed ends (fusobacteria)
6. Gram-negative curved rods (here commashaped vibrios)
7. Gram-negative diplococci, adjacent sides flattened (neisseria)
8. Gram-negative straight rods with rounded ends (coli bacteria)
9. Spiral rods (spirilla) and Gram-negative curved rods (*Helicobacter*)
10. Peritrichous flagellation
11. Lophotrichous flagellation
12. Monotrichous flagellation
13. Formation of endospores (sporulation) in cells of the genera *Bacillus* and *Clostridium* (spore stain)
    a) Central spore, vegetative cell shows no swelling
    b) Terminal spore, vegetative cell shows no swelling
    c) Terminal spore ("tennis racquet")
    d) Central spore, vegetative cell shows swelling
    e) Terminal spore ("drumstick")
14. Free spores (spore stain)

Table 3.**1** Morphological Characteristics of Bacteria (see Fig. 3.**1** for examples)

Bacterial form	Remarks
Cocci	Occur in clusters (Fig. 3.**2**), chains, pairs (diplococci), packets
Straight rods	Uniform thickness, rounded ends (Fig. 3.**3**), pointed ends, club form
Curved rods	Commashaped, spiral (Fig. 3.**4**), screwshaped
Mycoplasmas	Bacteria without a rigid cell wall; coccoid cells, long threads
Chlamydiae	Two forms: spherical/oval elementary bodies (300 nm); spherical/oval reticulate bodies (1000 nm)
Rickettsiae	Short coccoid rods (0.3–1 µm)

■ **Simple staining.** In this technique, a single staining substance, e.g., methylene blue, is used.

■ **Differential staining.** Two stains with differing affinities to different bacteria are used in differential staining techniques, the most important of which is gram staining. Gram-positive bacteria stain blue-violet, Gram-negative bacteria stain red (see p. 211 for method).

Three basic forms are observed in bacteria: spherical, straight rods, and curved rods (see Figs. 3.**1**–3.**4**).

Fine Structures of Bacteria

Nucleoid (Nucleus Equivalent) and Plasmids

The "cellular nucleus" in prokaryotes consists of a tangle of double-stranded DNA, not surrounded by a membrane and localized in the cytoplasm (Fig. 3.**5**). In *E. coli* (and probably in all bacteria), it takes the form of a single circular molecule of DNA. The genome of *E. coli* comprises 4.63×10^6 base pairs (bp) that code for 4288 different proteins. The genomic sequence of many bacteria is known.

The plasmids are nonessential genetic structures. These circular, twisted DNA molecules are 100–1000× smaller than the nucleoid genome structure and reproduce autonomously (Fig. 3.**6**). The plasmids of human pathogen bacteria often bear important genes determining the phenotype of their cells (resistance genes, virulence genes).

Cocci

Fig. 3.**2** Cocci are spherical bacteria. Those found in grapelike clusters as in this picture are staphylococci (Scanning electron microscopy (SEM)).

Rod Bacteria

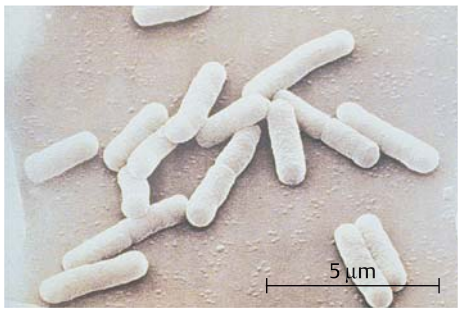

Fig. 3.**3** The straight rod bacteria with rounded ends shown here are coli bacteria (SEM).

Spirilla

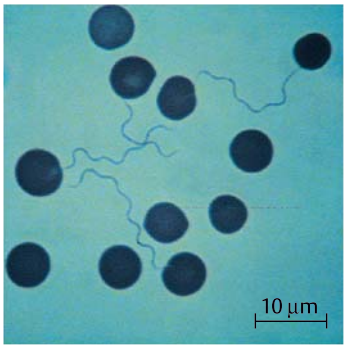

Fig. 3.**4** Spirilla, in this case borrelia are spiral bacteria (light microscopy (LM), Giemsa stain).

Bacteria During Cell Division

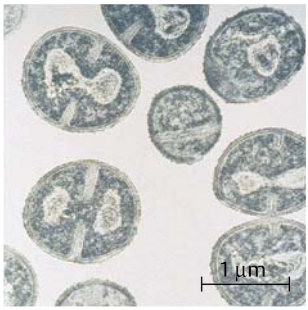

Fig. 3.**5** The nucleoid (nucleus equivalent) of bacteria consists of a tangled circular DNA molecule without a nuclear membrane. Transmission electron microscopy (TEM) image of staphylococci.

DNA Topology in Bacterial Cells

The DNA double helix (one winding/10 base pairs) is also wound counterclockwise about its helical axis (one winding/15 helical windings). This so-called supercoiling is necessary to save space and energy. Only supercoiled DNA can be replicated and transcribed. Topoisomerases steer the supercoiling process. DNA gyrase and topoisomerase IV are topoisomerases that occur only in bacteria. The 4-quinolones, an important group of anti-infection substances, inactivate these enzymes irreversibly.

Plasmids

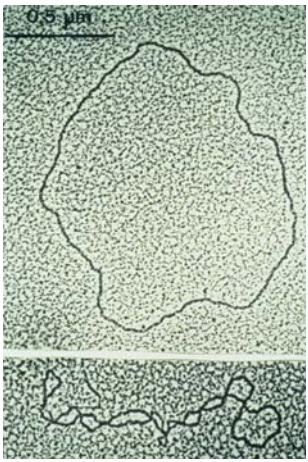

Fig. 3.**6** **a** Open circular form (OC). The result of a rupture in one of the two nucleic acid strands.
b Twisted (CCC = covalently closed circular), native form (TEM image).

a

b

Cytoplasm

The cytoplasm contains a large number of solute low- and high-molecular-weight substances, RNA and approximately 20 000 ribosomes per cell. Bacteria have 70S ribosomes comprising 30S and 50S subunits. Bacterial ribosomes function as the organelles for protein synthesis. The cytoplasm is also frequently used to store reserve substances (glycogen depots, polymerized metaphosphates, lipids).

The Most Important Bacterial Cytoplasmic Membrane Proteins

Permeases	Active transport of nutrients from outside to inside against a concentration gradient.
Biosynthesis enzymes	Required for biosynthesis of the cell wall, e.g., its murein (see under "Cell wall" p. 152). The enzymes that contribute to the final murein biosynthesis steps are for the most part identical with the "penicillin-binding proteins" (PBPs).
Secretion system proteins	Four secretion systems differing in structure and mode of action have been described to date. Proteins are moved out of the cell with the help of these systems. A common feature of all four is the formation of protein cylinders that traverse the cytoplasmic membrane and, in Gram-negative bacteria, the outer cell wall membrane as well. See p. 17 on the special relevance of the type III secretion system to virulence.
Sensor proteins (also known as signal proteins)	Transmit information from the cell's environment into its interior. The so-called receiver domain extends outward, the transmitter domain inward. The transmission activity is regulated by the binding of signal molecules to a receiver module. In two-component systems, the transmitter module transfers the information to a regulator protein, activating its functional module. This regulator segment can then bind to specific gene sequences and activate or deactivate one or more genes (see also Fig. 1.**4**, p. 19).
Respiratory chain enzymes	Occur in bacteria with aerobic metabolism. Aerobic respiration functions according to the same principles as cellular respiration in eurkaryotes.

The Cytoplasmic Membrane

This elementary membrane, also known as the plasma membrane, is typical of living cells. It is basically a double layer of phospholipids with numerous proteins integrated into its structure. The most important of these membrane

Basic Bacterial Cell Structure

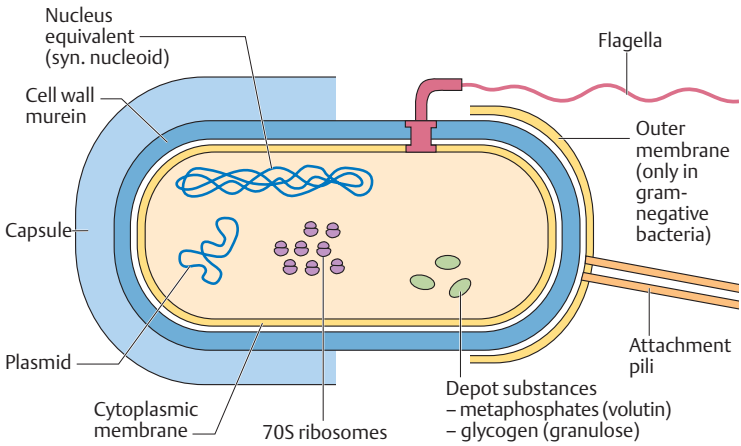

Fig. 3.**7** All bacteria have the same basic structure (not to scale).

proteins are permeases, enzymes for the biosynthesis of the cell wall, transfer proteins for secretion of extracellular proteins, sensor or signal proteins, and respiratory chain enzymes.

In electron microscopic images of Gram-positive bacteria, the mesosomes appear as structures bound to the membrane. How they function and what role they play remain to be clarified. They may be no more than artifacts.

Cell Wall

The tasks of the complex bacterial cell wall are to protect the protoplasts from external noxae, to withstand and maintain the osmotic pressure gradient between the cell interior and the extracellular environment (with internal pressures as high as 500–2000 kPa), to give the cell its outer form and to facilitate communication with its surroundings.

Murein (syn. peptidoglycan). The most important structural element of the wall is murein, a netlike polymer material surrounding the entire cell (sacculus). It is made up of polysaccharide chains crosslinked by peptides (Figs. 3.**8** and 3.**9**).

The cell wall of Gram-positive bacteria (Fig. 3.**10**). The murein sacculus may consist of as many as 40 layers (15–80 nm thick) and account for as much as

The Murein Building Block

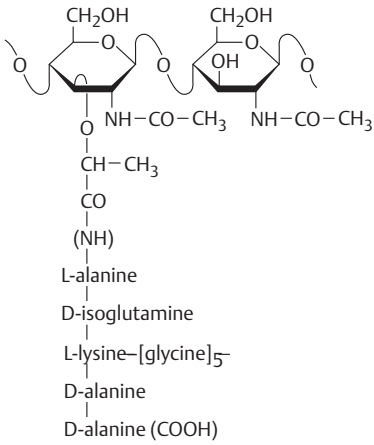

Fig. 3.**8** The murein (syn. peptidoglycan) of the cell wall is composed of a series of identical subunits. The terminal D-alanine is split off each time a new crosslink is synthesized. Only in staphylococci is a pentaglycine interpeptide bridge inserted between adjacent peptides.

30% of the dry mass of the cell wall. The membrane lipoteichoic acids are anchored in the cytoplasmic membrane, whereas the cell wall teichoic acids are covalently coupled to the murein. The physiological role of the teichoic

The Structure of Murein

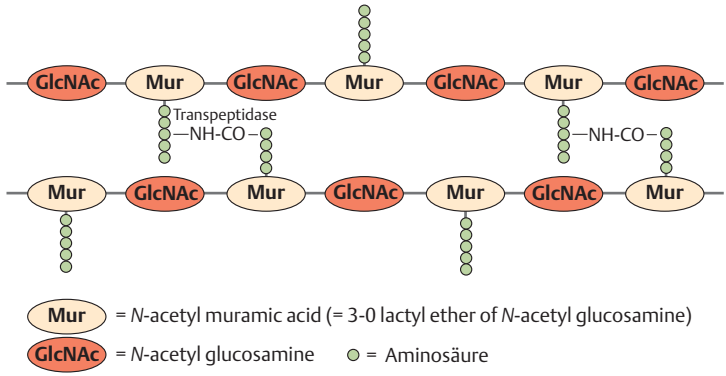

Fig. 3.**9** Soluble murein fragments of Gram-negative and Gram-positive bacteria can stimulate excessive cytokine secretion in macrophages by binding to toll-like receptors and CD14. Cytokines cause the clinical symptoms of sepsis or septic shock syndrome (see under Lipoid A, p. 156).

The Cell Wall of Gram-Positive Bacteria

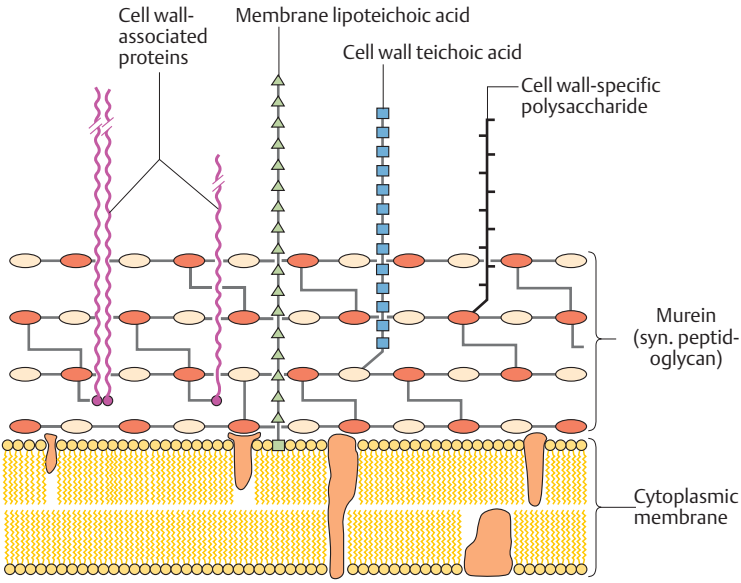

Fig. 3.**10** Note the characteristic thick murein layer, the proteins and teichoic acids anchored in the murein, and the lipoteichoic acid fixed to the membrane by a lipophilic anchor (not to scale).

acids is not known in detail; possibly they regulate the activity of the autolysins that steer growth and transverse fission processes in the cell. Within the macroorganism, teichoic acids can activate the alternative complement pathway and stimulate macrophages to secrete cytokines. Examples of cell wall-associated proteins are protein A, the clumping factor, and the fibronectin-binding protein of *Staphylococcus aureus* or the M protein of *Streptococcus pyogenes*. Cell wall anchor regions in these proteins extending far beyond the murein are bound covalently to its peptide components. Cell wall-associated proteins frequently function as pathogenicity determinants (specific adherence; phagocyte protection).

The cell wall of Gram-negative bacteria. Here, the murein is only about 2 nm thick and contributes up to 10% of the dry cell wall mass (Fig. 3.**11**). The outer membrane is the salient structural element. It contains numerous proteins (50% by mass) as well as the medically critical lipopolysaccharide.

The Cell Wall of Gram-Negative Bacteria

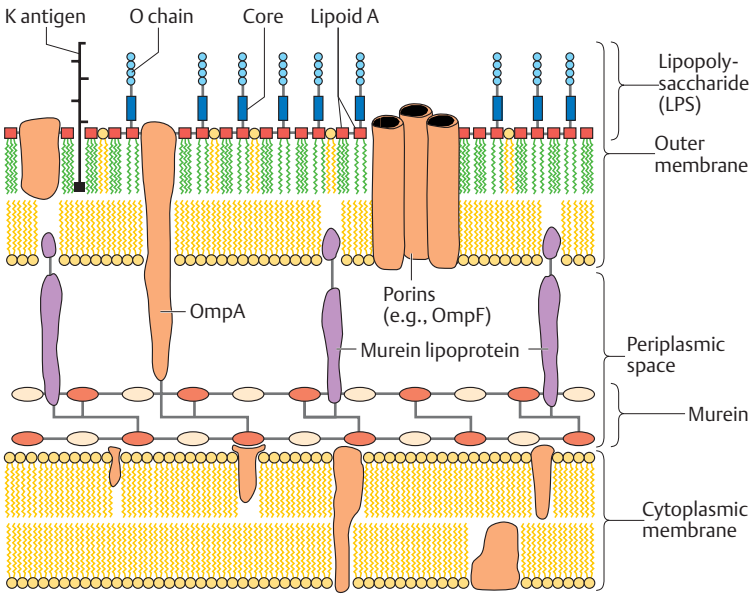

Fig. 3.**11** Note the characteristic thin murein layer and the outer membrane connected to it by proteins (OmpA, murein lipoprotein). Many different proteins are localized in the outer membrane. Its outer layer is made up of closely packed lipopolysaccharide complexes (see Fig. 3.12).

■ **Outer membrane proteins.**
- OmpA (outer membrane protein A) and the murein lipoprotein form a bond between outer membrane and murein.
- Porins, proteins that form pores in the outer membrane, allow passage of hydrophilic, low-molecular-weight substances into the periplasmic space.
- Outer membrane-associated proteins constitute specific structures that enable bacteria to attach to host cell receptors.
- A number of Omps are transport proteins. Examples include the LamB proteins for maltose transport and FepA for transport of the siderophore ferric (Fe^{3+}) enterochelin in *E. coli* (see also p. 13).

■ **Lipopolysaccharide (LPS).** This molecular complex, also known as endotoxin, is comprised of the lipoid A, the core polysaccharide, and the O-specific polysaccharide chain (Fig. 3.**12**).

The Lipopolysaccharide Complex

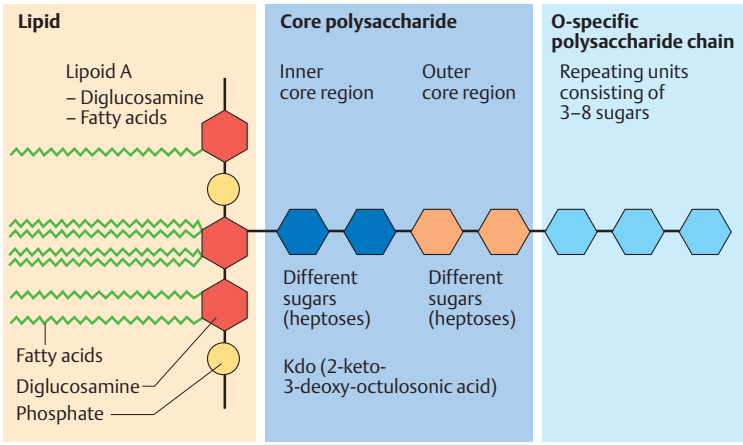

Fig. 3.**12** The three-part lipopolysaccharide complex (LPS) of Gram-negative bacteria is anchored in the outer membrane by means of its lipid moiety. LPS is also known as endotoxin.

– **Lipoid A** is responsible for the toxic effect. As a free substance, or bound up in the LPS complex, it stimulates—by binding together with the LPS binding protein (LBP) to the CD14 receptor of macrophages—the formation and secretion of cytokines that determine clinical endotoxin symptomatology. Interleukin 1 (IL-1) and tumor necrosis factor (TNF) induce an increased synthesis of prostaglandin E2 in the hypothalamus, thus setting the "thermostat" in the temperature control center higher, resulting in fever. Other direct and indirect endotoxin effects include granulopoiesis stimulation, aggregation and degeneration of thrombocytes, intravasal coagulation due to factor VII activation, a drop in blood pressure, and cachexia. LPS can also activate the alternative complement pathway. Release of large amounts of endotoxin can lead to septic (endotoxic) shock. Endotoxin is not inactivated by vapor sterilization. Therefore, the parent materials used in production of parenteral pharmaceuticals must be free of endotoxins (pyrogens).
– **The O-specific polysaccharide chain** is the so-called O antigen, the fine chemical structure of which results in a large number of antigenic variants useful in bacterial typing (e.g., detailed differentiation of salmonella types) (see p. 284f.).

L-forms (L = Lister Institute). The L-forms are bacteria with murein defects, e.g., resulting from the effects of betalactam antibiotics. L-forms are highly

unstable when subjected to osmotic influences. They are totally resistant to betalactams, which block the biosynthesis of murein. The clinical significance of the L-forms is not clear. They may revert to the normal bacterial form when betalactam therapy is discontinued, resulting in a relapse.

Capsule

Many pathogenic bacteria make use of extracellular enzymes to synthesize a polymer that forms a layer around the cell: the capsule. **The capsule protects bacterial cells from phagocytosis.** The capsule of most bacteria consists of a polysaccharide. The bacteria of a single species can be classified in different capsular serovars (or serotypes) based on the fine chemical structure of this polysaccharide.

Flagella

Flagella give bacteria the ability to move about actively. The flagella (singular flagellum) are made up of a class of linear proteins called flagellins. Flagel-

Bacterial flagella

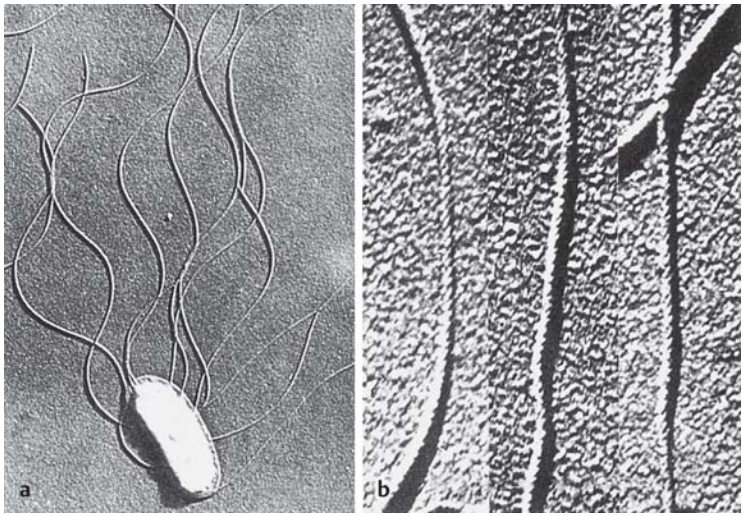

Fig. 3.**13** **a** Flagellated bacterial cell (SEM, 13 000×). **b** Helical structure of bacterial flagella (SEM, 77 000×).

lated bacteria are described as monotrichous, lophotrichous, or peritrichous, depending on how the flagella are arranged (see Fig. 3.**1**, p. 147). The basal body traverses the cell wall and cytoplasmic membrane to anchor the flagellum (see Figs. 3.**7** and 3.**13**) and enables it to whirl about its axis like a propeller. In *Enterobacteriaceae*, the flagellar antigens are called H antigens. Together with the O antigens, they are used to classify bacteria in serovars.

Attachment Pili (Fimbriae), Conjugation Pili

Many Gram-negative bacteria possess thin microfibrils made of proteins (0.1–1.5 nm thick, 4–8 nm long), the attachment pili. They are anchored in the outer membrane of the cell wall and extend radially from the surface. Using these structures, bacteria are capable of specific attachment to host cell receptors (ligand—receptor, key—keyhole).

The conjugation pili (syn. sex pili) in Gram-negative bacteria are required for the process of conjugation and thus for transfer of conjugative plasmids (see p. 175).

Examples of Attachment Pili in Gram-Negative Bacteria

PAP (syn. P pili)	Pyelonephritis-associated pili. Bind to receptors of the uroepithelium and to the P blood group antigen (hence "P" pili). The specific receptors for these pili are plentiful on the uroepithelial surface. PAP are characteristic of the uropathological variety of *Escherichia coli* that causes spontaneous urinary tract infections in patients showing no tract obstruction.
CFA1, CFA2	Colonization factors. Pili responsible for specific binding of enteropathogenic coli bacteria to enterocytes.
Gonococcal attachment pili	Used for specific attachment of gonococci mucosal cells of the urogenital epithelium.

Biofilm

A bacterial biofilm is a structured community of bacterial cells embedded in a self-produced polymer matrix and attached to either an inert surface or living tissue. Such films can develop considerable thickness (mm). The bacteria located deep within such a biofilm structure are effectively isolated from immune system cells, antibodies, and antibiotics. The polymers they secrete are frequently glycosides, from which the term glycocalyx (glycoside cup) for the matrix is derived.

Examples of Medically Important Biofilms

- Following implantation of endoprostheses, catheters, cardiac pacemakers, shunt valves, etc. these foreign bodies are covered by matrix proteins of the macroorganism such as fibrinogen, fibronectin, vitronectin, or laminin. Staphylococci have proteins on their surfaces with which they can bind specifically to the corresponding proteins, for example the clumping factor that binds to fibrinogen and the fibronectin-binding protein. The adhering bacteria then proliferate and secrete an exopolysaccharide glycocalyx: the biofilm matrix on the foreign body. Such biofilms represent **foreign body-associated infection foci**.
- Certain oral streptococci (*S. mutans*) bind to the proteins covering tooth enamel, then proceed to build a glucan matrix out of sucrose. Other bacteria then adhere to the matrix to form plaque (Fig. 3.**14**), the precondition for destruction of the enamel and formation of **caries** (see p. 243f.).
- Oral streptococci and other bacteria attach to the surface of the cardiac valves to form a biofilm. Professional phagocytes are attracted to the site and attempt, unsuccessfully, to phagocytize the bacteria. The frustrated phagocytes then release the tissue-damaging content of their lysosomes (see p. 23), resulting in an inflammatory reaction and the clinical picture of **endocarditis**.

Bacterial Spores

Bacterial spores (endospores) are purely dormant life forms. Their development from bacterial cells in a "vegetative" state does not involve assimilation of additional external nutrients. They are spherical to oval in shape and are characterized by a thick spore wall and a high level of resistance to chemical and physical noxae. Among human pathogen bacteria, only the genera *Clostridium* and *Bacillus* produce spores. The heat resistance of these spores is their most important quality from a medical point of view, since heat ster-

Dental Plaque

Fig. 3.**14** Dental plaque can be rendered visible with an erythrosin stain.

ilization procedures require very high temperatures to kill them effectively. Potential contributing factors to spore heat resistance include their thick wall structures, the dehydration of the spore, and crosslinking of the proteins by the calcium salt of pyridine-2,6-dicarboxylic acid, both of which render protein denaturing difficult. When a spore's milieu once again provides favorable conditions (nutrient medium, temperature, osmotic pressure, etc.) it returns to the vegetative state in which spore-forming bacteria can reproduce.

The Physiology of Metabolism and Growth in Bacteria

Human pathogenic bacteria are chemosynthetic and organotrophic (chemo-organotrophic). They derive energy from the breakdown of organic nutrients and use this chemical energy both for resynthesis and secondary activities. Bacteria oxidize nutrient substrates by means of either respiration or fermentation. In respiration, O_2 is the electron and proton acceptor, in fermentation an organic molecule performs this function. Human pathogenic bacteria are classified in terms of their O_2 requirements and tolerance as facultative anaerobes, obligate aerobes, obligate anaerobes, or aerotolerant anaerobes. Nutrient broth or agar is used to cultivate bacteria. Nutrient agar contains the inert substrate agarose, which liquefies at 100 °C and gels at 45 °C. Selective and indicator mediums are used frequently in diagnostic bacteriology.

Bacteria reproduce by means of simple transverse binary fission. The time required for complete cell division is called generation time. The in-vitro generation time of rapidly proliferating species is 15–30 minutes. This time is much longer in vivo. The growth curve for proliferation in nutrient broth is normally characterized by the phases lag, log (or exponential) growth, stationary growth, and death.

Bacterial Metabolism

Types of Metabolism

Metabolism is the totality of chemical reactions occurring in bacterial cells. They can be subdivided into anabolic (synthetic) reactions that consume energy and catabolic reactions that supply energy. In the anabolic, endergonic

reactions, the energy requirement is consumed in the form of light or chemical energy—by photosynthetic or chemosynthetic bacteria, respectively. Catabolic reactions supply both energy and the basic structural elements for synthesis of specific bacterial molecules. Bacteria that feed on inorganic nutrients are said to be lithotrophic, those that feed on organic nutrients are organotrophic.

Human pathogenic bacteria are always chemosynthetic, organotrophic bacteria (or chemo-organotrophs).

Catabolic Reactions

Organic nutrient substrates are catabolized in a wide variety of enzymatic processes that can be schematically divided into four phases:

Digestion. Bacterial exoenzymes split up the nutrient substrates into smaller molecules outside the cell. The exoenzymes represent important pathogenicity factors in some cases.

Uptake. Nutrients can be taken up by means of passive diffusion or, more frequently, specifically by active transport through the membrane(s). Cytoplasmic membrane permeases play an important role in these processes.

Preparation for oxidation. Splitting off of carboxyl and amino groups, phosphorylation, etc.

Oxidation. This process is defined as the removal of electrons and H^+ ions. The substance to which the H_2 atoms are transferred is called the hydrogen acceptor. The two basic forms of oxidation are defined by the final hydrogen acceptor (Fig. 3.**15**).

- **Respiration.** Here oxygen is the hydrogen acceptor. In anaerobic respiration, the O_2 that serves as the hydrogen acceptor is a component of an inorganic salt.
- **Fermentation.** Here an organic compound serves as the hydrogen acceptor.

The main difference between fermentation and respiration is the energy yield, which can be greater from respiration than from fermentation for a given nutrient substrate by as much as a factor of 10. Fermentation processes involving microorganisms are designated by the final product, e.g., alcoholic fermentation, butyric acid fermentation, etc.

The energy released by oxidation is stored as chemical energy in the form of a thioester (e.g., acetyl-CoA) or organic phosphates (e.g., ATP).

Bacterial Oxidation Pathways

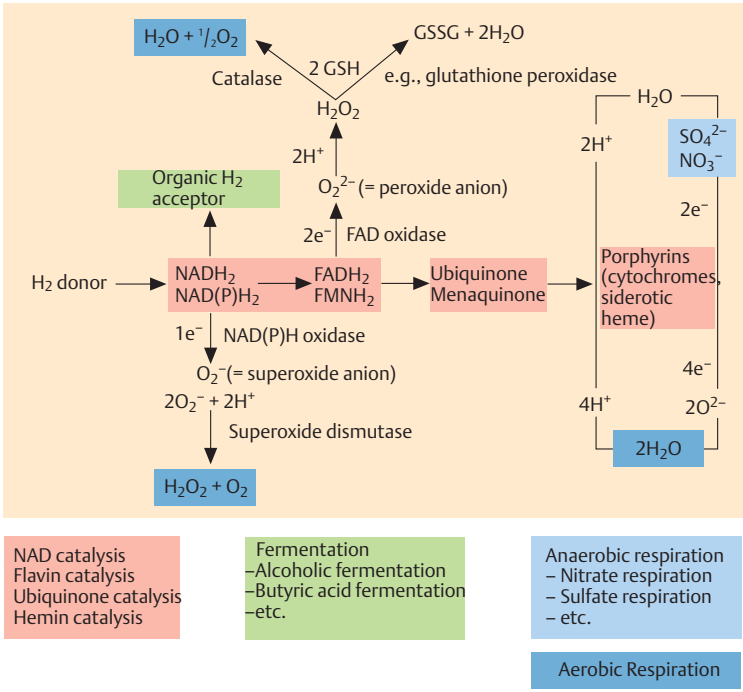

Fig. 3.**15** In oxidation of organic nutrient substrates, protons (H^+) and electrons (e^-) are transferred in more or less long chains. The respiration is aerobic when the final electron acceptor is free oxygen. Anaerobic respiration is when the electrons are transferred to inorganically bound oxygen. Fermentation is the transfer of H^+ and e^- to an organic acceptor.

The role of oxygen. Oxygen is activated in one of three ways:

- Transfer of $4e^-$ to O_2, resulting in two oxygen ions ($2\ O^{2-}$).
- Transfer of $2e^-$ to O_2, resulting in one peroxide anion ($1\ O_2^{2-}$).
- Transfer of $1e^-$ to O_2, resulting in one superoxide anion ($1\ O_2^-$).

Hydrogen peroxide and the highly reactive superoxide anion are toxic and therefore must undergo further conversion immediately (see Fig. 3.**15**).

Bacteria are categorized as the following according to their O_2-related behavior:

- **Facultative anaerobes.** These bacteria can oxidize nutrient substrates by means of both respiration and fermentation.
- **Obligate aerobes.** These bacteria can only reproduce in the presence of O_2.
- **Obligate anaerobes.** These bacteria die in the presence of O_2. Their metabolism is adapted to a low redox potential and vital enzymes are inhibited by O_2.
- **Aerotolerant anaerobes.** These bacteria oxidize nutrient substrates without using elemental oxygen although, unlike obligate anaerobes, they can tolerate it.

Basic mechanisms of catabolic metabolism. The principle of the biochemical unity of life asserts that all life on earth is, in essence, the same. Thus, the catabolic intermediary metabolism of bacteria is, for the most part, equivalent to what takes place in eukaryotic cells. The reader is referred to textbooks of general microbiology for exhaustive treatment of the pathways of intermediary bacterial metabolism.

Anabolic Reactions

It is not possible to go into all of the biosynthetic feats of bacteria here. Suffice it to say that they are, on the whole, quite astounding. Some bacteria (*E. coli*) are capable of synthesizing all of the complex organic molecules that they are comprised of, from the simplest nutrients in a very short time. These capacities are utilized in the field of microbiological engineering. Antibiotics, amino acids, and vitamins are produced with the help of bacteria. Some bacteria are even capable of using aliphatic hydrocarbon compounds as an energy source. Such bacteria can "feed" on paraffin or even raw petroleum. It is hoped that the metabolic capabilities of these bacteria will help control the effects of oil spills in surface water. Bacteria have also been enlisted in the fight against hunger: certain bacteria and fungi are cultivated on aliphatic hydrocarbon substrates, which supply carbon and energy, then harvested and processed into a protein powder (single cell protein). Culturing of bacteria in nutrient mediums based on methanol is another approach being used to produce biomass.

Metabolic Regulation

Bacteria are highly efficient metabolic regulators, coordinating each individual reaction with other cell activities and with the available nutrients as economically and rationally as possible. One form such control activity takes is regulation of the activities of existing enzymes. Many enzymes are allosteric proteins that can be inhibited or activated by the final products of metabolic pathways. One highly economical type of regulation controls the synthesis of enzymes at the genetic transcription or translation level (see the section on the molecular basis of bacterial genetics (p. 169ff.).

Growth and Culturing of Bacteria

Nutrients

The term bacterial culture refers to proliferation of bacteria with a suitable nutrient substrate. A nutrient medium (Table 3.**2**) in which chemoorganotrophs are to be cultivated must have organic energy sources (H_2 donors) and H_2 acceptors. Other necessities include sources of carbon and nitrogen for synthesis of specific bacterial compounds as well as minerals such as sulfur, phosphorus, calcium, magnesium, and trace elements as enzyme activators. Some bacteria also require "growth factors," i.e., organic compounds they are unable to synthesize themselves. Depending on the bacterial species involved, the nutrient medium must contain certain amounts of O_2 and CO_2 and have certain pH and osmotic pressure levels.

Table 3.**2** Nutrient Mediums for Culturing Bacteria

Nutrient medium	Description
Nutrient broth	Complex liquid nutrient medium.
Nutrient agar	Complex nutrient medium containing the polysaccharide agarose (1.5–2%). Nutrient agar liquefies when heated to 100 °C and does not return to the gel state until cooled to 45 °C. Agarose is not broken down by bacteria.
Selective mediums	Contain inhibitor substances that allow only certain bacteria to proliferate.
Indicator mediums	Indicate certain metabolic processes.
Synthetic mediums	Mediums that are precisely chemically defined.

Growth and Cell Death

Bacteria reproduce asexually by means of simple transverse binary fission. Their numbers (n) increase logarithmically ($n = 2^G$). The time required for a reproduction cycle (G) is called the generation time (g) and can vary greatly from species to species. Fast-growing bacteria cultivated in vitro have a generation time of 15–30 minutes. The same bacteria may take hours to reproduce in vivo. Obligate anaerobes grow much more slowly than aerobes; this is true in vitro as well. Tuberculosis bacteria have an in-vitro generation time of 12–24 hours. Of course the generation time also depends on the nutrient content of the medium.

The so-called **normal growth curve** for bacteria is obtained by inoculating a nutrient broth with bacteria the metabolism of which is initially quiescent, counting them at intervals and entering the results in a semilog coordinate system (Fig. 3.**16**). The lag phase (A) is characterized by an increase in bacterial mass per unit of volume, but no increase in cell count. During this phase, the metabolism of the bacteria adapts to the conditions of the nutrient medium. In the following log (or exponential) phase (C), the cell count increases logarithmically up to about 10^9/ml. This is followed by growth deceleration and transition to the stationary phase (E) due to exhaustion of the nutrients and the increasing concentration of toxic metabolites. Finally, death phase (F) processes begin. The generation time can only be determined during phase C, either graphically or by determining the cell count (n) at two different times and applying the formula:

$$g = \frac{t_2 - t_1}{\log_2 n_2 - \log_2 n_1} .$$

Normal Growth Curve of a Bacterial Culture

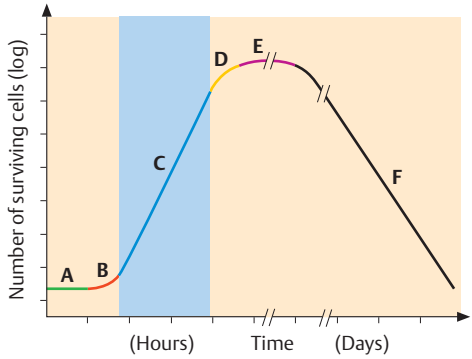

Fig. 3.**16** A = lag phase, B = acceleration phase, C = log (exponential) phase, D = deceleration phase, E = stationary phase, F = death phase.

Bacterial Cell Count and Bacterial Mass

The colony counting method. The number of living cells in a given culture or material can be determined by means of the colony counting method. The samples are diluted logarithmically by a dilution factor of 10. Using the pour plate technique, each dilution is mixed with 1 ml of liquid agar and poured out in a plate. In the surface inoculation method, 0.1 ml of each dilution is plated out on a nutrient agar surface. The plates are incubated, resulting in colony growth. The number of colonies counted, multiplied by the dilution factor, results in the original number of viable bacterial cells (CFU = colony forming units).

Bacterial mass. The bacterial mass can be established by weighing (dry or wet weight). The simplest way to determine the mass is by means of photometric adsorption measurement. The increases in mass and cell count run parallel during phase C on the growth curve.

The Molecular Basis of Bacterial Genetics

Bacteria possess two genetic structures: the **chromosome** and the **plasmid**. Both of these structures consist of a single circular DNA double helix twisted counterclockwise about its helical axis. **Replication** of this DNA molecule always starts at a certain point (the origin of replication) and is "semiconservative," that is, one strand in each of the two resulting double strands is conserved. Most **bacterial genes** code for proteins (polypeptides). Noncoding interposed sequences (introns), like those seen in eukaryotes, are the exception. Certain bacterial genes have a mosaic structure. The phases of **transcription** are promoter recognition, elongation, and termination. Many bacterial mRNAs are polycistronic, meaning they contain the genetic information for several polypeptides. **Translation** takes place on the 70S ribosomes. Special mRNA codons mark the start and stop of polypeptide synthesis. Many genes that code for functionally related polypeptides are grouped together in chromosome or plasmid segments known as operons. The most important regulatory mechanism is the positive or negative control of transcription initiation. This control function may be exercised by individual localized genes, the genes of an operon or genes in a regulon.

The Structure of Bacterial DNA

A bacterium's genetic information is stored in its chromosome and plasmids. Each of these structures is made of a single DNA double helix twisted to the right, then additionally twisted to the left about its helical axis (supercoiled, see p. 148ff. and Fig. 3.**17**). Plasmids consisting of linear DNA also occur, although this is rare. This DNA topology solves spatial problems and enables such functions as replication, transcription, and recombination. Some genes are composed of a mosaic of minicassettes interconnected by conserved DNA sequences between the cassettes (see Fig. 1.**2**, p. 14).

Chromosome. The chromosome corresponds to the nucleoid (p. 148ff.). The *E. coli* chromosome is composed of 4.63×10^6 base pairs (bp). It codes for 4288 proteins. The gene sequence is colinear with the expressed genetic products. The noncoding interposed sequences (introns) normally seen in eukaryotic genes are very rare. The chromosomes of E. coli and numerous other pathogenic bacteria have now been completely sequenced.

Plasmids. The plasmids are autonomous DNA molecules of varying size (3×10^3 to 4.5×10^5 bp) localized in the cytoplasm. Large plasmids are usually present in one to two copies per cell, whereas small ones may be present in 10, 40, or 100 copies. Plasmids are not essential to a cell's survival.

Resistance Plasmid in *Escherichia coli*

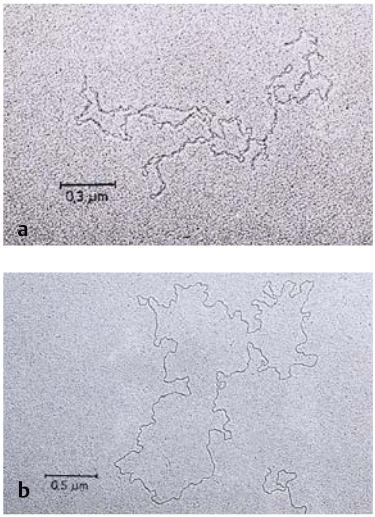

Fig. 3.**17** **a** Covalently closed circle (CCC), also known as a "supercoil" or "supertwist."
b Open circle. This open form is an artifact produced by a nick in one strand of the DNA double helix.

Many of them carry genes that code for certain phenotypic characteristics of the host cell. The following plasmid types are medically relevant:

- **Virulence plasmids.** Carry determinants of bacterial virulence, e.g., enterotoxin genes or hemolysin genes.
- **Resistance plasmids.** Carry genetic information bearing on resistance to anti-infective agents. R plasmids may carry several *R* genes at once (see also Fig. 3.**23**, p. 176). Plasmids have also been described that carry both virulence and resistance genes.

DNA Replication

The identical duplication process of DNA is termed semiconservative because the double strand of DNA is opened up during replication, whereupon each strand serves as the matrix for synthesis of a complementary strand. Thus each of the two new double strands "conserves" one old strand. The doubling of each DNA molecule (replicon) begins at a given starting point, the so-called origin of replication. This process continues throughout the entire fission cycle.

Transcription and Translation

- **Transcription.** Copying of the sense strand of the DNA into mRNA. The continuous genetic nucleotide sequence is transcribed "colinearly" into mRNA. This principle of colinearity applies with very few exceptions. The transcription process can be broken down into the three phases *promoter recognition*, *elongation*, and *termination*. The promoter region is the site where the RNA polymerase begins reading the DNA sequence. A sigma factor is required for binding to the promoter. Sigma factors are proteins that associate temporarily with the RNA polymerase (core enzyme) to form a holoenzyme, then dissociate themselves once the transcription process has begun, making them available to associate once again. Specific sigma factors recognize the standard promoters of most genes. Additional sigma factors, the expression of which depends on the physiological status of the cell, facilitate the transcription of special determinants. Genes that code for functionally related proteins, for example proteins that act together to catalyze a certain metabolic step, are often arranged sequentially at specific locations on the chromosome or plasmid. Such DNA sequences are known as operons (Fig. 3.**18**). The mRNA synthesized by the transcription of an operon is polycistronic, i.e., it contains the information sequences of several genes. The information sequences are

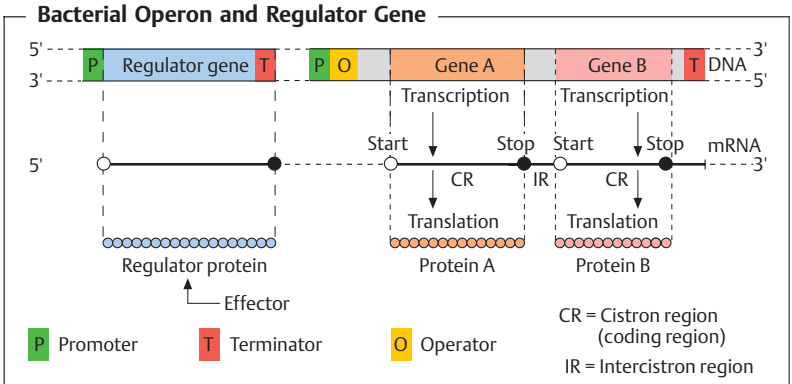

Fig. 3.**18** An operon is a DNA sequence that usually includes several structural genes. These genes code for proteins that are functionally related. Transcription of an operon is often activated or repressed by the product of a regulator gene located elsewhere on the chromosome.

separated by intercistronic regions. Each cistron has its own start and stop codon in the mRNA.

Translation. Transformation of the nucleotide sequence carried by the mRNA into the polypeptide amino acid sequence at the 70S ribosomes. In principle, bacterial and eukaryotic translation is the same. The enzymes and other factors involved do, however, differ structurally and can therefore be selectively blocked by antibiotics (p. 198ff.).

Regulation of Gene Expression

Bacteria demonstrate a truly impressive capacity for adapting to their environment. A number of regulatory bacterial mechanisms are known, for example posttranslational regulation, translational regulation, transcription termination, and quorum sensing (see Fig. 1.**5**, p. 20). The details of all these mechanisms would exceed the scope of this book. The most important is regulation of the initiation of transcription by means of activation or repression, a process not observed in this form in eukaryotes: a single gene, or several genes in an operon at one DNA location, may be affected (see Fig. 3.**18**). The mechanism that has been investigated most thoroughly is transcriptional regulation of catabolic and anabolic operons by a repressor or activator.

Transcriptional Regulation of an Operon:

- **Catabolic operons** have genes that code for enzymes of catabolic metabolism. Anabolic operons code for enzymes of anabolic metabolism.
- **Regulators.** Code for proteins that can repress or activate transcription by binding to the operator or promoter of an operon.
- **Effectors.** Low-molecular-weight signal molecules from the immediate environment of the bacterial cell. Can activate (= corepressor) or inactivate (= inducer) the repressor by means of an allosteric effect.
- **Induction of a catabolic operon.** The effector molecule is a nutrient substrate that is broken down by the products of the operon genes (e.g., lactose). Lactose inactivates the repressor, initializing transcription of the genes for *β-galactosidase* and *β-galactoside permease* in the lactose operon. These genes are normally not read off because the repressor is bound to the operator. The cell is not induced to produce the necessary catabolic enzymes until the nutrient substrate is present.
- **Repression of an anabolic operon.** The signal molecule is the final product of an anabolic process, for instance an amino acid. If this acid is present in the medium, it can be obtained from there and the cell need not synthesize the anabolic enzymes it would require to produce it. In such a case, binding to the effector is what turns the regulator protein into an active repressor.

A single regulator protein can also activate or repress several genes not integrated in an operon, i.e., at various locations on the DNA. Such functional gene groups are called **regulons**. Alternative **sigma factors** (see p. 168) may be involved in the transcriptional activation of special genes with special promoters. Physiological cell status determines whether or not these alternative factors are produced.

The Genetic Variability of Bacteria

Changes in bacterial DNA are the result of spontaneous **mutations** in individual genes as well as recombination processes resulting in new genes or genetic combinations. Based on the molecular mechanisms involved, bacterial **recombinations** are classified as homologous, site-specific, and transpositional. The latter two in particular reflect the high level of mobility of many genes and have made essential contributions to the evolution of bacteria.

Although sexual heredity is unknown in bacteria, they do make use of the mechanisms of intercellular transfer of genomic material known as parasexual processes. **Transformation** designates transfer of DNA that is essentially

chemically pure from a donor into a receptor cell. In **transduction**, bacteriophages serve as the vehicles for DNA transport. **Conjugation** is the transfer of DNA by means of cell-to-cell contact. This process, made possible by conjugative plasmids and transposons, can be a high-frequency one and may even occur between partners of different species, genera, or families. The transfer primarily involves the conjugative elements themselves. Conjugative structures carrying resistance or virulence genes are of considerable medical significance.

The processes of **restriction** and **modification** are important factors limiting genetic exchange among different taxa. Restriction is based on the effects of restriction endonucleases capable of specific excision of foreign DNA sequences. These enzymes have become invaluable tools in the field of genetic engineering.

Molecular Mechanisms of Genetic Variability

Spontaneous Mutation

In the year 1943, Luria and Delbrück used the so-called fluctuation test to demonstrate that changes in the characteristics of bacterial populations were the results of rare, random mutations in the genes of individual cells, which then were selected. Such mutations may involve substitution of a single nucleotide, frame-shifts, deletions, inversions, or insertions. The frequency of mutations is expressed as the **mutation rate**, which is defined as the probability of mutation per gene per cell division. The rate varies depending on the gene involved and is approximately 10^{-6} to 10^{-10}. Mutation rates may increase drastically due to mutagenic factors such as radioactivity, UV radiation, alkylating chemicals, etc.

Recombination

The term recombination designates processes that lead to the restructuring of DNA, formation of new genes or genetic combinations.

Homologous (generalized) recombination. A precise exchange of DNA between corresponding sequences. Several enzymes contribute to the complex breakage and reunion process involved, the most important being the RecA enzyme and another the RecBC nuclease. Fig. 1.**2** (p. 14) shows an example of homologous recombination resulting in the exchange of minicassettes between two genes.

Site-specific recombination. Integration or excision of a sequence in or from target DNA. Only a single sequence of a few nucleotides of the integrated DNA needs to be homologous with the recombination site on the target DNA. The integration of bacteriophage genomes is an example of what this process facilitates (p. 184f.) Integration of several determinants of antibiotic resistance in one integron can also utilize this process (Fig. 3.**19**). Resistance integrons may be integrated in transposable DNA.

Transposition. The transposition process does not require the donor and target DNA to be homologous. DNA sequences can either be transposed to a different locus on the same molecule or to a different replicon. Just as in site-specific recombination, transposition has always played a major role in the evolution of multi-resistance plasmids (see Fig. 3.**23**, p. 176).

Site-Specific Recombination (Integron)

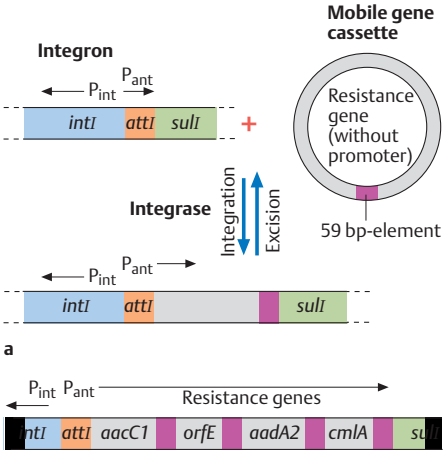

Fig. 3.**19** **a** Integration or excision of a circular gene cassette into or out of an integron. An integron is a genetic structure containing the determinants of a site-specific recombination system. This structure is capable of capturing or mobilizing mobile gene cassettes. It also provides the promoter for transcription of the cassette genes, which themselves have no promoter. The cassettes occur in both free circularized and integrated forms.

b

intI	*Integrase* gene. Codes for site-specific integrase (int = integrase).
attI	Attachment sequence for the integrase. Site of recombination (att = attachment).
P_{ant}	Promoter for the integrated gene cassette (P = promoter).
P_{int}	Promoter for the *integrase* gene.
sulI	Gene for sulfonamide resistance (sul = sulfonamide).
59bp	Integrase-specific recombination site in cassette.

b Model of integron 4 (In*4*), which contains three resistance genes and an open reading frame (*orfE*), the function of which is unknown. In*4* is the result of successive integration of several resistance genes at the *att1* site.

Transposable DNA Elements

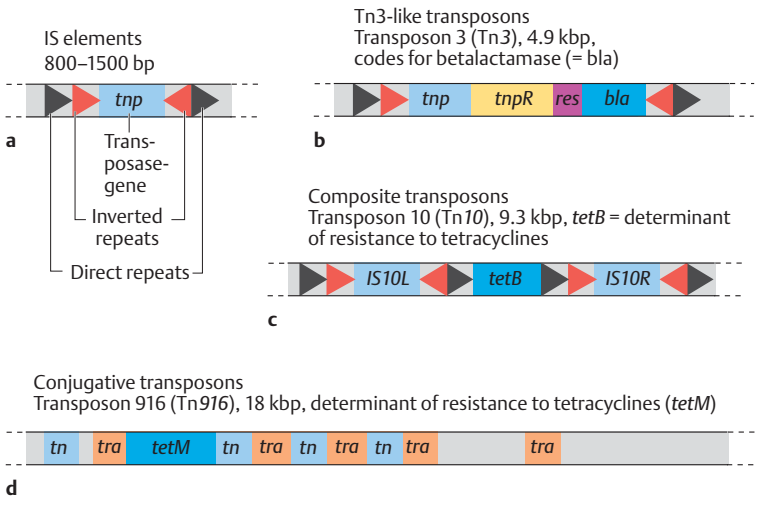

Fig. 3.**20** **a–d** Explanation of the structures and abbreviations used in the text box "Details of Transposable DNA elements" below.

Details of Transposable DNA Elements

- **Insertion sequences (IS elements**, Fig. 3.**20**). These are the simplest transposable DNA sequences. They are terminated by identical, but reversed, sequences of 10–40 nucleotides known as inverted repeats (IR). They frame the segment that codes for the enzyme transposase. The target structures for this enzyme are the so-called *direct repeats*, nucleotide sequences comprising 5–9 bp that are duplicated in the integration process.

- **Tn3 transposons** (Fig. 3.**20b**). In addition to the transposase gene *tnpA*, they contain the regulator sequence *tnpR* and the *res* site to which resolvase must bind. Tn3-like transposons are duplicated in the transposition process, so that one copy remains at the original location and the other is integrated at the new location.

- **Composite transposons** (Fig. 3.**20c**). They consist of two IS elements framing a sequence of variable size that is not required for transposition, e.g., a resistance gene.

- **Conjugative transposons** (Fig. 3.**20d**). These genetic elements code in certain regions for factors that control the transfer (Tra) and transposition (Tn) processes. Conjugative transposons have been discovered mainly in Gram-positive cocci and Gram-negative anaerobes (*Bacteroides*).

Intercellular Mechanisms of Genetic Variability

Although bacteria have no sexual heredity in the strict sense, they do have mechanisms that allow for intercellular DNA transfer. These mechanisms, which involve a unilateral transfer of genetic information from a donor cell to a receptor cell, are subsumed under the term **parasexuality**.

Transformation

Transfer of "naked" DNA. In 1928, Griffith demonstrated that the ability to produce a certain type of capsule could be transferred between different pneumococci. Then Avery showed in 1944 that the transforming principle at work was DNA. This transformation process has been observed mainly in the genera *Streptococcus*, *Neisseria*, *Helicobacter* and *Haemophilus*.

Transduction

Transfer of DNA from a donor to a receptor with the help of transport bacteriophages (Fig. 3.**21**).

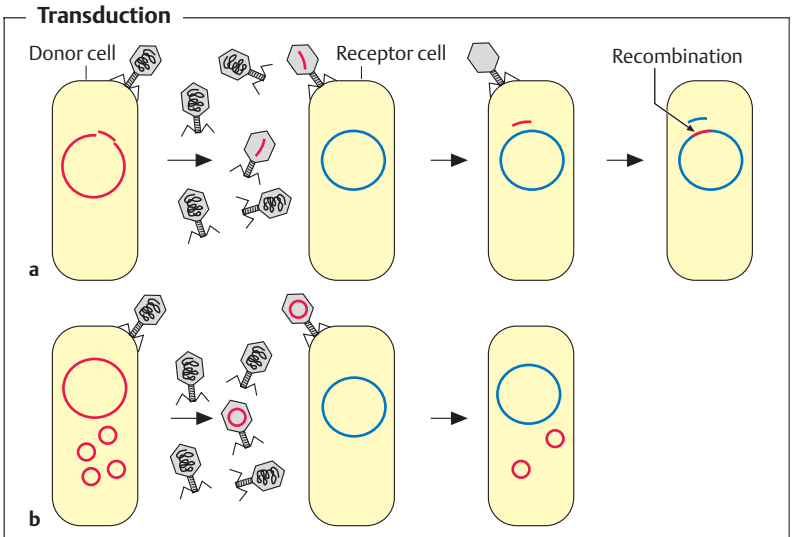

Fig. 3.**21** Transduction of a chromosomal DNA sequence (**a**) and a plasmid (**b**).

Bacteriophages are viruses that infect bacteria (p. 182ff.). During their replication process, DNA sequences from the host bacterial cell may replace all or part of the genome in the phage head. Such phage particles are then defective. They can still dock on receptor cells and inject their DNA, but the infected bacterial cell will then neither produce new phages nor be destroyed.

Conjugation

Conjugation is the transfer of DNA from a donor to a receptor in a conjugal process involving cell-to-cell contact. Conjugation is made possible by two genetic elements: the conjugative plasmids and the conjugative transposons.

Conjugation

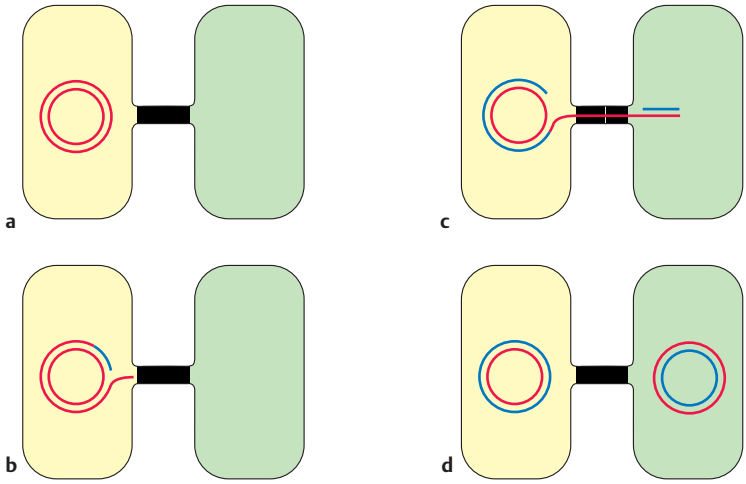

Fig. 3.**22** Transfer/replication process of a conjugative plasmid.
a Conjugation: connection between two bacterial cells by means of sex pili. This initial step alone does not necessarily always lead to effective conjugation.
b Effective conjugation: formation of a specific conjugal bridge between donor cell and receptor cell.
c Plasmid mobilization and transfer: an endonuclease cleaves one strand of the circular DNA double helix at a specific point (**b**). The single strand with the "leader region" enters the receptor cell.
d Synthesis: the double-stranded structure of both the transferred single strand and the remaining DNA strand is restored by means of complementary DNA synthesis. The receptor cell, now plasmid-positive, is called a transconjugant.

In the conjugation process, the conjugative elements themselves are what are primarily transferred. However, these elements can also mobilize chromosomal genes or otherwise nontransferable plasmids. Conjugation is seen frequently in Gram-negative rods (*Enterobacteriaceae*), in which the phenomenon has been most thoroughly researched, and enterococci.

The F-factor in *Escherichia coli*. This is the prototype of a conjugative plasmid. This factor contains the so-called *tra* (transfer) genes responsible both for

Model of a Hypothetical Conjugative Multiple-Resistance Plasmid

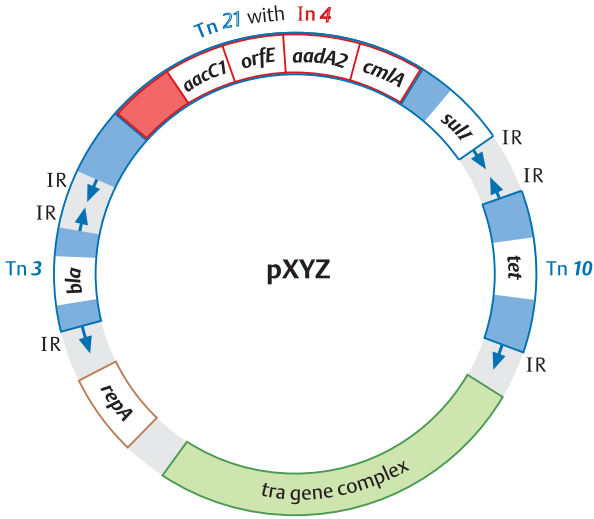

Fig. 3.**23** Multiple resistance plasmids can result from successive integration of transposable resistance DNA or integration of resistance integrons (see Figs. 3.**19** and 3.**20**, p. 173 and 174)

Tn*21* Transposon of the Tn*21* family, codes for resistance to sulfonamides (*sulI*) and contains an R integron (In*4*).

In*4* Codes for chloramphenicol acetyltransferase (= *cmlA*), an aminoglycoside acetyltransferase (= *aacC1*) and an aminoglycoside adenylyltransferase (= *aadA2*); also contains an open reading frame (*orfE*) of unknown function.

Tn*3* Transposon 3; codes for a betalactamase (= *bla*).

Tn*10* Transposon 10; codes for resistance to tetracyclines (= *tet*).

repA Codes for the replication enzyme of the plasmid.

tra Plasmid DNA region containing 25 *tra* genes; *tra* genes are responsible for the transfer and replication process (see Fig. 3.**22**).

the formation of conjugal pili on the surface of F cells and for the transfer process. The transfer of the conjugative plasmid takes place as shown here in schematic steps (Fig. 3.**22**).

Occasional integration of the F factor into the chromosome gives it the conjugative properties of the F factor. Such an integration produces a sort of giant conjugative element, so that chromosomal genes can also be transferred by the same mechanism. Cells with an integrated F factor are therefore called Hfr ("high frequency of recombination") cells.

Conjugative resistance and virulence plasmids. Conjugative plasmids that carry determinants coding for antibiotic resistance and/or virulence in addition to the *tra* genes and *repA* are of considerable medical importance. Three characteristics of conjugative plasmids promote a highly efficient horizontal spread of these determinant factors among different bacteria:

- **High frequency of transfer.** Due to the "transfer replication" mechanism, each receptor cell that has received a conjugative plasmid automatically becomes a donor cell. Each plasmid-positive cell is also capable of multiple plasmid transfers to receptor cells.

- **Wide range of hosts.** Many conjugative plasmids can be transferred between different taxonomic species, genera, or even families.

- **Multiple determinants.** Many conjugative plasmids carry several genes determining the phenotype of the carrier cell. The evolution of a hypothetical conjugative plasmid carrying several resistance determinants is shown schematically in Fig. 3.**23**.

Conjugative transposons. These are DNA elements (p. 173) that are usually integrated into the bacterial chromosome. They occur mainly in Gram-positive cocci, but have also been found in Gram-negative bacteria (*Bacteroides*). Conjugative transposons may carry determinants for antibiotic resistance and thus contribute to horizontal resistance transfer. In the transfer process, the transposon is first excised from the chromosome and circularized. Then a single strand of the double helix is cut and the linearized single strand—analogous to the F factor—is transferred into the receptor cell. Conjugative transposons are also capable of mobilizing nonconjugative plasmids.

Restriction, Modification, and Gene Cloning

The above descriptions of the mechanisms of genetic variability might make the impression that genes pass freely back and forth among the different bacterial species, rendering the species definitions irrelevant. This is not the case. A number of control mechanisms limit these genetic exchange processes. Among the most important are **restriction** and **modification**. Re-

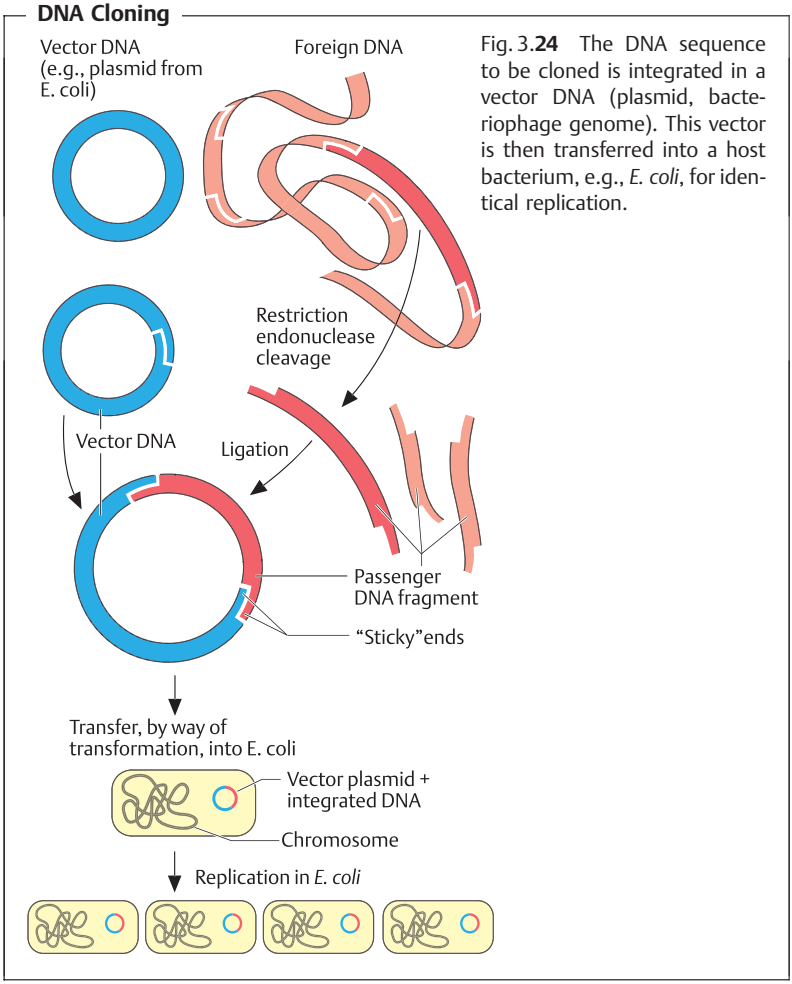

Fig. 3.**24** The DNA sequence to be cloned is integrated in a vector DNA (plasmid, bacteriophage genome). This vector is then transferred into a host bacterium, e.g., *E. coli*, for identical replication.

striction endonucleases can destroy foreign DNA that bears no "fingerprint" (modification) signifying "self." These modifications take the form of methylation of the DNA bases by modification enzymes.

Bacterial restriction endonucleases are invaluable tools in modern **gene cloning** techniques. The process is termed gene "cloning" because it involves replication of DNA that has been manipulated in vitro in a suitable host cell so as to produce identical copies of this DNA: molecular clones or gene clones.

The technique simplifies the replication of DNA, making experimental manipulations easier. On the other hand, the bacteria can also be used to synthesize gene products of the foreign genes. Such foreign proteins are called recombinant proteins. Bacterial plasmids often function in the role of vectors into which the sequences to be cloned are inserted. Fig. 3.**24** illustrates the principle of gene cloning in simplified form.

Table 3.**3** lists the most important terms used in the field of bacterial genetics.

Table 3.**3** Glossary of Important Terminology in Bacterial Genetics

Anticodon	Triplet sequences of transfer RNA complementary to the codons of mRNA
Chromosome	See nucleoid
Cistron	Genetic unit, identical to "gene"
Code	Key relating the DNA nucleotide (n = 3) sequence to the polypeptide amino acid sequence
Codon	Sequence of three nucleotides, triplet
Corepressor	See effector molecules
Deletion	Loss of a DNA sequence in a replicon
Effector molecules	Small molecules that inactivate (= inductor) or activate (= corepressor) a regulator protein by means of an allosteric effect
Episome	Historical term; characterizes a replicon (e.g., F plasmid) occurring either in the cytoplasm or integrated in the bacterial chromosome
F factor	Prototype of a conjugative plasmid (fertility factor)
Gene	DNA segment containing the information used in synthesis of a polypeptide or RNA
Genome	All of the genetic information contained in a cell
Genotype	The totality of genetically determined characteristics
Hfr cells	Coli bacteria with F factor integrated into their chromosomes, therefore capable of transferring chromosomal genes at a high frequency by means of conjugation (Hfr = high frequency of recombination)
Inductor	See effector molecules
Integron	Genetic structure containing the determinants for a site-specific recombination system; responsible for integration or excision of mobile gene cassettes

Table 3.**3** *Continued: Glossary of Important Terminology. . .*

Inverted repeats	Nucleotide sequences repeated in reverse order at the ends of transposable DNA
IS	Insertion sequences; transposable DNA elements
Cassette	Sequence in a gene that can be transferred to other genes by homologous recombination
Clone	Population of identical cells or DNA molecules
Conjugation	Transfer of hereditary material in a pairing process
Lysogenic bacteria	Cells with a phage genome (prophage) integrated into their chromosomes
Lysogenic conversion	Change in cell phenotype brought about by prophage genes
Messenger RNA	Synthesized at the DNA by transcription; carries genetic information to the ribosomes
Modification enzymes	Methylases that label DNA as "self" by methylation
Mutation	A permanent alteration of the genome
Nucleoid	Nuclear region, nucleus equivalent
Operator	DNA sequence of an operon; regulator binding site
Operon	Regulatory unit comprising the promoter, operator, structural genes, and terminator
Parasexuality	Unilateral gene transfer from a donor to a receptor
Phenotype	The totality of characteristics expressed in a bacterial cell
Plasmid	Extrachromosomal, autonomous, in most cases circular DNA molecule
Promoter	Recognition and binding site for RNA polymerase
Prophage	Phage genome integrated into the chromosome
Regulator	Regulatory protein that controls gene transcription; repressor or activator
Regulon	Functional unit of genes at different loci controlled by the same regulator
Recombination, Legitimate or homologous recombination	Replacement of a DNA sequence by a homologous sequence from a different genome; breakage and reunion model

Table 3.**3** *Continued: Glossary of Important Terminology. . .*

Recombination, Illegitimate recombination	Insertion of transposable DNA
Site-specific recombination	Integration or excision of a DNA sequence by means of homologous recombination in a specific DNA segment comprising only a small number of nucleotides
Replication	Reproduction, duplication of DNA
Replicon	DNA molecule that replicates autonomously
Restriction endonucleases	Enzymes that recognize and cleave specific DNA nucleotide sequences
Semiconservative replication	DNA duplication mechanism in which one old strand is conserved in each of the two new double strands
Conjugal (or sex) pili	Surface structures essential to conjugation in Gram-negative rod bacteria
Sigma factors	Proteins that temporarily associate with prokaryotic RNA polymerase for specific promoter binding
Supercoil	Circular DNA molecule additionally twisted about the helical axis in the opposite direction
Terminator	Sequence marking the end of a transcription process
Transduction	Gene transfer using bacteriophages as vehicles
Transfer RNA	Specifically binds an amino acid (aminoacyl tRNA) and transfers it to the ribosome
Transformation	Transfer of genes from a donor in the form of "naked" DNA
Transcription	RNA synthesis at DNA
Translation	Ribosomal synthesis of polypeptides
Transposase	Transposition enzyme; facilitates illegitimate recombination
Transposition	Translocation of a mobile DNA element within a replicon or between different replicons
Transposon	Transposable DNA; frequently contains—in addition to the genes for transposition—determinants that change the phenotype of a bacterial cell
Triplet code	Three nucleotides coding for one amino acid
Vector	Vehicle for foreign (passenger) DNA; usually a plasmid or phage genome