2 The Structure and Ultrastructure of the Cell

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The structure and living manifestation of cells are the realm of **cell biology**. This discipline unites ultrastructure (microstructure, fine structure) research, biochemistry, and molecular biology, as well as many aspects of physiology. Before 1950, before the modern methods of cell research had been established, the study of cells was called **cytology** (Greek *kýtos*, bubble, cell). It was mainly restricted to the light microscopy of cells.

Cell research is hugely significant because all forms of life are organized in cells. Many organisms are **unicellular**, where a single cell represents the individual. This is true for most prokaryotes and, according to definition, also for all eukaryotic protists, including flagellates from various algal divisions and diatoms. Eukaryotes are mostly **multicellular**. As the cells are generally microscopically small, large multicellular organisms have achieved unbelievably high numbers of cells. A tree can be made up of more than ten trillion cells. Even a medium-sized leaf is made up of about 20 million cells.

A. Bresinsky et al., *Strasburger's Plant Sciences*, DOI 10.1007/978-3-642-15518-5_2,

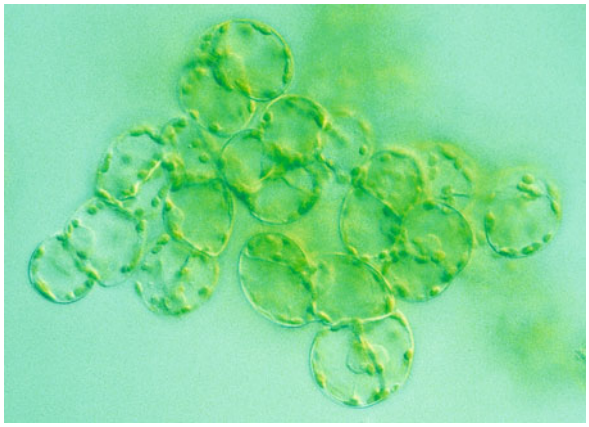

Fig. 2.1
One-week-old autotrophic suspension culture of *Glycine max* (soybean) cells (×120). Some cells have already divided once or more times. This can be used for "cloning" or artificial production of genetically uniform (identical) plant material

Multicellular organisms are phylogenetically younger than unicellular organisms. During the evolution of multicellular organisms, a number of significant vital functions have remained fixed at the single-cell level. This is especially true for the storage, replication, realization, and recombination of genetic information. Nearly every cell in a body contains a cell nucleus with the complete, often usually diploid, gene or chromosome complement. The cell can double this gene complement by DNA replication and distribute identical copies to daughter cells (**mitosis**; see Sect. 2.2.3.5). All the body cells of a multicellular organism generally share the same gene complement, making them part of a cell clone. It seems therefore paradoxical that during their individual development (**ontogeny**) cells nevertheless differentiate in a regular fashion, taking on different forms and functions. This differentiation or determination problem has basically been resolved since it is understood that each stage of differentiation is a result of the activation of a characteristic part of the gene complement and the repression of the remaining genes. Activation and repression of genes are controlled by integration signals that, as long as they do not have an environmental origin causing individual adaptation, come from certain cells (in a multicellular system) and are reacted to by other specialized cells.

Sexual processes can only occur in the single-cell state. Special gametes are formed in this case. The most biologically significant sexual processes are **meiosis** with recombination and **syngamy** (cell and nucleus fusion of genetically different gametes of the same species) (see Sects. 2.2.3.7–2.2.3.9).

Cells can only arise from like cells by division or fusion: "*omnis cellula* e *cellula*" (R. Virchow 1858). The characteristics of living systems listed in the Introduction are only manifested at the cell level, not below. Thus, a cell is the smallest unit capable of life – an **elementary organism**. That this is also true for multicellular organisms can be seen in the sexual processes; it is also proven by the fact that it is possible to culture cells (*Fig. 2.1*).

2.1 Cell Biology

The development of cell research is a good example of the dependency of advancements in natural sciences on methodological possibilities. Most cells are microscopically small. The first descriptions of cells date from the seventeenth century after the invention of the microscope. The fundamental similarity of plant, animal, and protist cells was only discovered after microscopes had undergone significant improvements at the start of the nineteenth century.

Once the cell nucleus had been observed in plant tissue and then also in animal and human cells, T. Schwann published his epochal work *Microscopic Investigations on the Accordance in the Structure and Growth of Plants and Animals* in 1839. Thus, the first pillar of general biology was established. Further observations based on the consistent application of microscopic techniques and the first cell physiology investigations (e.g., osmosis) followed rapidly. During the second half of the nineteenth century, three main principles of cytology began to crystallize:

1. All living organisms are made up of cells.
2. Cells are the basic building units of life.
3. The individual development of the multicellular organism begins – at least with regard to sexual reproduction – with a unicellular stage.

Around 1880, after E. Abbe had made improvements to microscope optics, the theoretical seeing limit of 0.2 µm was reached. At the same time, the preparation techniques had also undergone important advances. By 1900, all cell organelles visible with a light microscope had been described (*Fig. 2.2*). After the rediscovery of Mendel's inheritance laws at the start of the twentieth century, the emphasis of research for the following four decades was mainly on cell nuclei and chromosomes (**karyology**, **cytogenetics**).

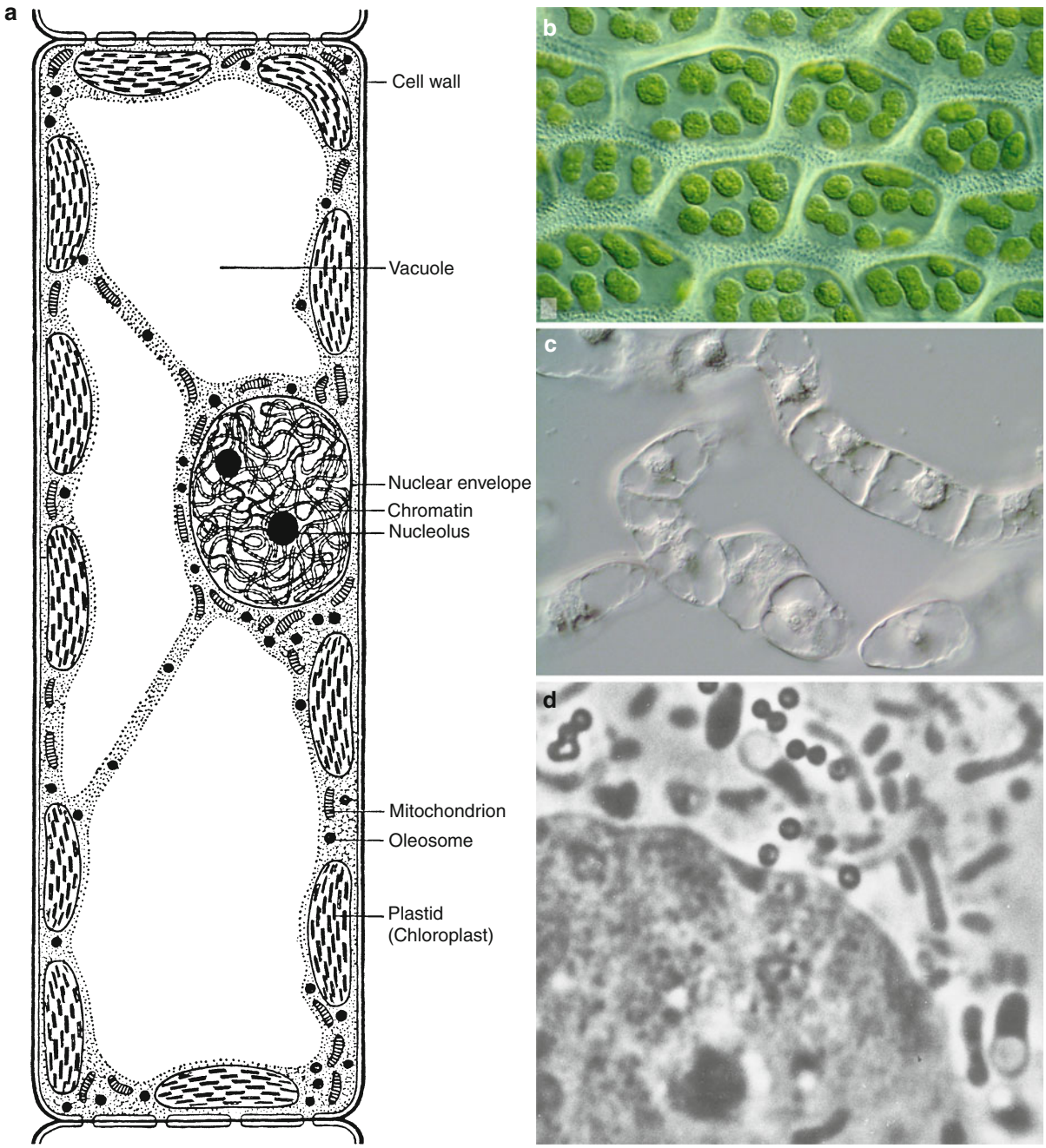

Fig. 2.2

The plant cell under the light microscope (LM). (a) A photosynthetic parenchyma cell of a deciduous leaf. (b) Chloroplasts in the cells of a leaf (*Katharina ondulata*, ×300). (c) Cells in a suspension culture (tobacco; BY2) (×350): the large cells are almost filled with the central vacuole; the cell nuclei and nucleoli are in the parietal cytoplasm tube that thickens at the cell corners; numerous other cytoplasm tubes running through the cell can also be seen. (d) Nuclear region of an *Allium* cell, phase contrast (×3,100); chromatin and a nucleolus are in the nucleus, leucoplasts (two with pale starch-like inclusions), sausage-shaped mitochondria, and spherical oleosomes are in the cytoplasm (a After D. von Denffer; b, c interference microscope images from P. Sitte; d phase contrast microscope image from P. Sitte)

The explosive development of cell research after 1945 – the **ultrastructural**, **biochemical**, and **molecular phase** – was again paralleled by technological advancements: electron microscopy, cell fractionation using an ultracentrifuge (Box 2.1), X-ray structure analysis of biomacromolecules. Recently, novel and quite diverse observation and preparation techniques have greatly expanded the potential for **studying living cells**. This is especially important in the transition from genomics to proteonomics.

Box 2.1: Cell Fractionation

The **ultracentrifuge** enables the harvesting of uniform fractions of subcellular particles for biochemical or analytical study (Fig. B2.1). The cell structure is lost in the process. Large quantities of uniform cells are carefully broken down into a suitable isolation medium, e.g., by mixing, crumbling, or using ultrasound. The resulting homogenized matrix ideally has no more entire cells but still has unbroken cell nuclei, plastids, and so on. The individual cell components can then be separated from the homogenized matrix.

Differential centrifugation is when the homogenized matrix is submitted to a series of centrifuge episodes of increasing acceleration (100–50,000 rpm; at high speeds, the centrifugal force can exceed that of Earth's acceleration due to gravity *g* by over 100,000 times). The fractionation is then according to particle weight or size. Initially, the nuclei and plastids are pelletized at low-speed revolutions (equivalent to $10^3 g$ for 10 min), i.e., they are sedimented out of the matrix. The pellet is resuspended as a more-or-less pure fraction after the rest of the centrifuged matrix has been poured off. The rest may be centrifuged again at higher speeds; the next fraction ($10^4 g$ for 30 min) would then contain mitochondria, for example.

Density gradient centrifugation results in the medium in the centrifugation tube separating out into layers of increasing density and concentration (from top to bottom) of saccharose, CsCl, or similar. In this case, the homogenized matrix particles are sorted according to their suspended density; each particle is positioned in the gradient, independently of size and weight, according to the matching density of the surrounding medium (isopycnic, or equilibrium, centrifugation).

The ultracentrifuge allows not only the sorting of subcellular particles but also sorting according to their characteristic ***S* number** ("*S*" after T. Svedberg, the inventor of the ultracentrifuge). These numbers refer to the particular sedimentation speed per centrifuge revolution in Svedberg units ($1S = 10^{-13}$ s) of a particle. The *S* number of spherical particles is proportional to $M^{2/3}$ (where *M* is the particle mass). In particular, ribosomes and their subparticles (general ribonucleoprotein particles) and protein complexes are typically characterized by their *S* number.

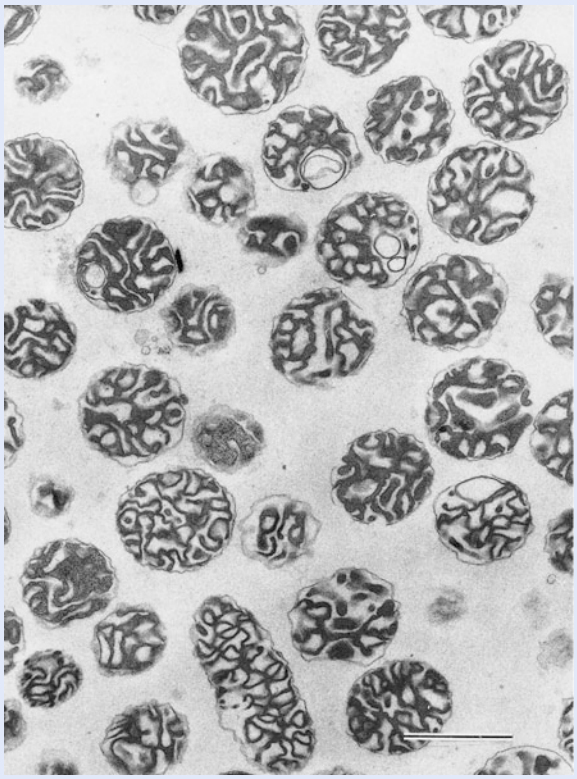

Fig. B2.1

Mitochondrial product from the isopycnic centrifugation of homogenized spinach tissue. The mitochondrial matrix has shrunk (compare with Fig. 2.78), and the compartmentalization has been retained. Impurities from other cell organs are negligible. *Scale bar* 1 μm (Specimen from B. Liedvogel; electron microscope, EM, image from H. Falk)

Further Reading

Graham J, Rickwood D (1997) Subcellular fractionation: a practical approach. IRL Press at Oxford University Press, Oxford

2.1.1 Light Microscopy

The lens of the **light microscope** (Fig. 2.3) gives an enlarged, photographable image of the object or specimens under study. This intermediate image is observed through the ocular (lens) just like looking through a magnifying glass. The smallest details visible with an optical resolution have to be at least 0.2 µm apart; macromolecular cell structures remain invisible. Even so, the light microscope has retained a certain advantage over higher-resolution electron microscopes because living material can be studied, and far less preparation is involved.

However, most cell structures are colorless and are hardly differentiated from their surroundings in terms of their refractive index. Thus, they often remain invisible even when their dimensions are larger than the minimum resolution. Therefore, classical light microscopy principally uses fixed specimens (dead but retaining the structure) and artificially stained specimens. Optically anisotropic cell structures such as cell walls, starch grains, and nuclear spindles can be seen in the living state using a **polarization microscope**, and their macromolecular structure can be analyzed. These days, the problem of contrast is solved by optical manipulation without affecting the specimen itself. Phase contrast or differential interference contrast techniques transform the phase differences in the light waves after they have passed through the specimen into contrast differences or apparent relief differences (Figs. 2.2c, d, 2.80, 3.9). The spatial presentation of cellular structures is possible using confocal laser scanning microscopy. Tomography is performed in microscopic dimensions; i.e., the specimen is (virtually) cut optically into a series of very thin slices that are then put together by a computer to generate a spatial image. This can then be observed on a screen from any angle desired. The individual images of the optical slices (they are scanned row by row in a rastering process) often give much clearer microscopic detail than can be seen on the specimen itself as disrupting layers are removed.

Cytochemical techniques are used to identify and localize certain molecules in a cell. Among these techniques, the especially sensitive fluorescence method is very important. In the **fluorescence microscope**, the specimen is irradiated with short-wavelength excitation radiation, and corresponding

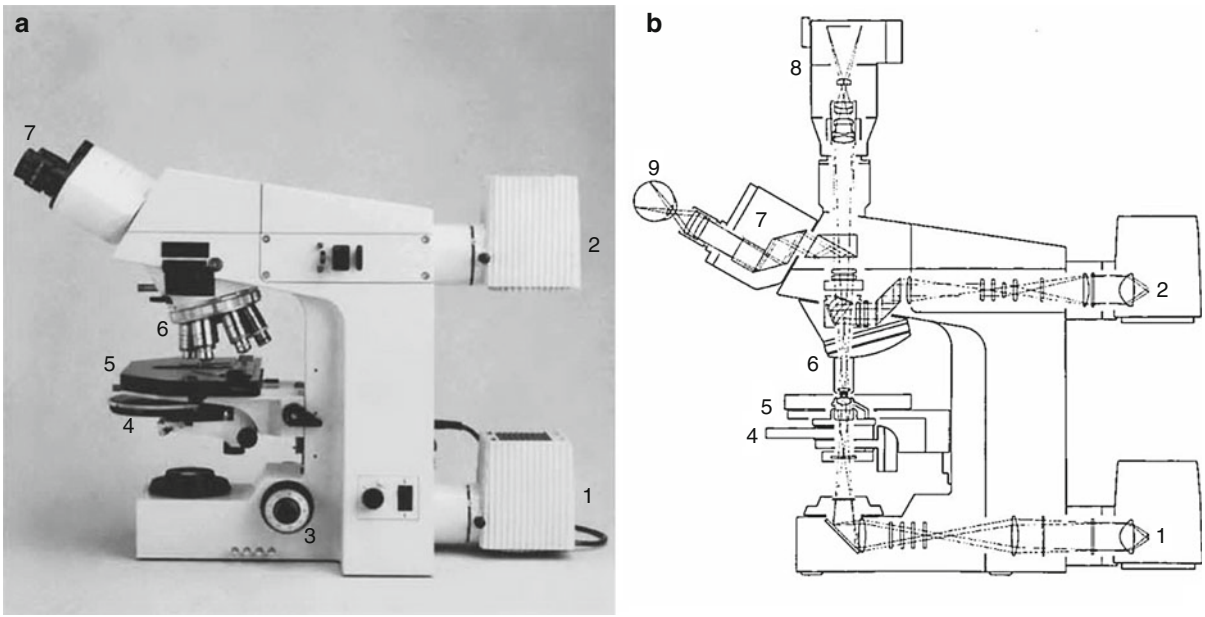

Fig. 2.3
Research microscope (Axioplan from Carl Zeiss) used for light microscopy. (a) Lateral view, seen from the left. (b) Beam entry: *1*, *2* light sources for transmission light and vertical illumination; *3* micrometer screw to adjust the focus by raising/lowering the stage, *5*; *4* condenser for light field illumination, phase contrast, and differential interference contrast; *6* objective turret revolver, above are slots for color and polarization filters including optical extensions; *7* (bin)ocular lens; *8* automatic microscope camera; *9* eye

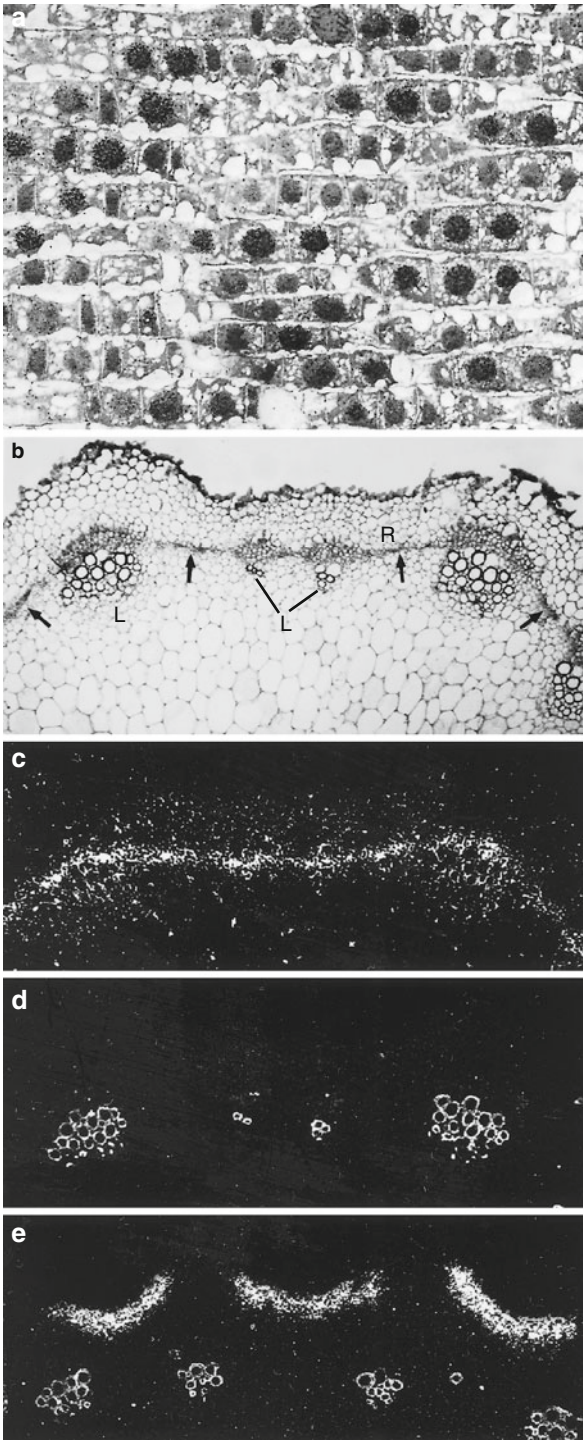

Fig. 2.4

Microradioautogram. (a) Root tip tissues of the common onion after pulse labeling with ^{3}H-thymidine. Cell nuclei whose DNA replicated during the pulse (S phase, see Sect. 2.2.3.5) are covered with lots of black grains of silver when the photoemulsion coating on the slide is developed. Unmarked nuclei were not in S phase during the pulse labeling. DNA-free cell structures are not marked by ^{3}H-thymidine (×380). (b–e) Evidence of transcripts (messenger RNAs, mRNAs) from in situ hybridization with synthetic, radioactive RNA probes in the transverse section of the shoot axis of mustard (*Sinapis alba*): (b) vascular bundles *L*, cortical tissues *R*, and shoot cambium, *arrows*; (d, e) different transcription activity of genes for an RNA-binding protein depending on the time of day (c greatest activity at the end of the light phase, cambium entirely marked; d lowest activity, no markings; the woody parts of the vascular bundle are illuminated in dark field even when not marked); (e) mRNA for a cell wall protein is only formed in the cells of the outer cortex (×60) (b–e Specimens and dark-field images from D. Staiger and C. Heintzen)

components of the specimen emit longer-wavelength fluorescent light. For production of the image, the excitation radiation is filtered out so that only the fluorescing objects are highlighted. Because only a few cell components actually fluoresce well, a series of techniques have been developed that target certain molecules, making them fluoresce. **Immunofluorescence** is of particular importance. The extreme specificity of mammalian antibody proteins is used to identify and precisely locate certain antigenic proteins, polysaccharides, or nucleic acids (see, e.g., *Fig. 2.10*). The last decade has seen the use of "**green fluorescent protein**" and its color variants as fluorescent markers revolutionize analytic microscopy (Zacharias and Tsien 2006). It allows the identification of gene activity in living cells, and certain proteins can be studied with regard to their occurrence, location, and behavior (see *Fig. 2.82c*). The diversity of fluorescence techniques complements the earlier technique of **microradioautography**. This incredibly sensitive method uses the ability of cells to incorporate radioactive isotopes in certain substances or structures, e.g., the tritium-labeled thymidine in DNA, its relative ^{3}H-uridine in RNA, or ^{35}S-methionine in proteins. After suitable dark-exposure times and developing of the thin-layer section containing the marked cells/tissue clusters, silver grains in the photographic emulsion coat mark the location of radionucleotides (*Fig. 2.4*).

Many investigations require the targeted manipulation of cells. Complex **micromanipulators** have been developed for this very task, although recently, laser tools ("**optical tweezers**") have gained in popularity.

As well as the new light microscope observation techniques, there are an array of other techniques that are important in modern cell biology. The cultivation of uniform cell clone lines

using **cell cultures** is fundamental (Fig. 2.1). The removal of the cell wall of **protoplasts** by the action of enzymes enables the application of a range of methods originally developed for animal and human cells. These include artificial **cell fusion** (Fig. 2.49) and **patch-clamp techniques** in order to investigate ion channels and receptors. In order to manipulate individual cells, the cell membrane often has to be punctured, at least temporarily and/or locally, so that the selected metabolic parameters (ion balance, pH value, energy levels, etc.) can be altered experimentally. **Permeabilized cells** (the cell membrane is rendered permeable with detergents) or so-called **cell models** (partially active cell remnants left after removal of the cell membrane that are no longer capable of independent survival) are used in this approach. Another alternative is given by the **microinjection** technique (see Fig. 2.48). Further possibilities for the introduction of macromolecules into living cells are provided by **electroporation** (short-term creation of permeable spots in the cell membrane by giving it an electrical impulse) and **biolistics** (about 1 µm diameter, gold or tungsten particles are coated with DNA or RNA and shot into, e.g., leaf tissue using a pressure wave). These methods enable, e.g., the blocking of particular enzymes in living cells by using introduced antibodies, and even genetic activity can be artificially altered using the targeted catapulting of foreign DNA (**transfection**), transcription factors, or antisense messenger RNA (mRNA) (see Sect. 6.2.2.3).

2.1.2 Electron Microscopy

In the **electron microscope** (Fig. 2.5), the illumination and imaging of objects is done using fast electrons that are refracted in the fields of electromagnetic lenses. The enlarged image is observed on a fluorescing screen and can be stored photographically or electronically. The wavelength of the electron beam is only 1/100,000 of that of visible light after acceleration with 100 kV. Thus, a much better resolution than with the light microscope can be achieved. The resolution is two orders of magnitude better for biological specimens.

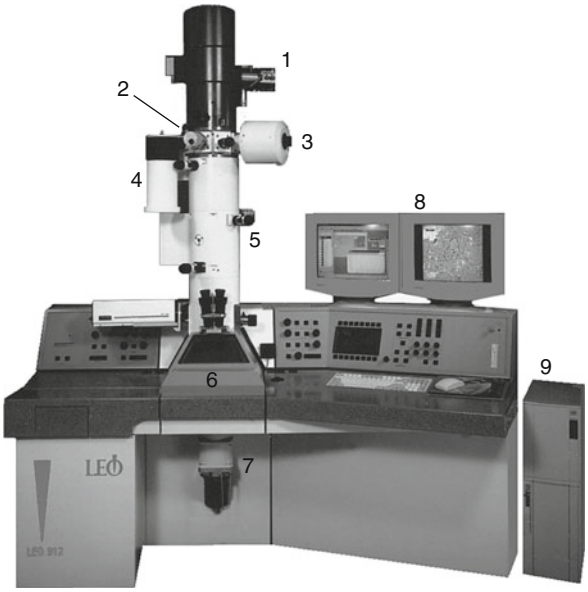

Fig. 2.5
Modern electron microscope (EM). The electron beams travel from the beam generator (electron gun, *1*) and pass through a condenser lens system (vertical tube *5*) from top to bottom, the specimen sitting in the strong vacuum of the tube (preparation hatch *2* with lateral Dewar flask *4* for liquid nitrogen to cool the specimen hatch; *3* motorized tip-tilt bench for the specimen), then the fields of the electromagnetic objective and projective imaging lenses (in *5*) and finally hit a fluorescent light screen. This final picture can be observed through a viewing window (*6*) or on monitors and can be photographed or stored digitally (digital camera *7*). The residual gas pressure in the tube is maintained by high-vacuum pumps to values of under one millionth of atmospheric pressure. *9* Computer tower for image acquisition and manipulation. The price of such a transmission electron microscope is more than €300,000 (LEO Elektronenmikroskopie, Oberkochen, Germany, model LEO 912)

Observations with a conventional **transmission electron microscope** (**TEM**) use specimens that can be no thicker than 80 nm; i.e., less than 1/1,000 the thickness of a sheet of paper. There are several processes by which specimens can be prepared for TEM observations. Transmitting particles (macromolecules, multienzyme complexes, DNA strands, ribosomes, viruses, cellulose fibrils, membrane fractions) are dried on the thinnest of plastic or carbon sheets and observed directly. To increase the contrast, heavy metals can be intercalated (positive contrast), attached (negative contrast; see, e.g., Figs. 1.16a, 1.17, 2.44, 2.65, 2.79), or obliquely vapor-deposited (shading with relief; Fig. 2.71). Cells and tissues are polymerized (embedded) in hard plastic (epoxy resin) after chemical fixation with glutaraldehyde and osmium tetroxide and cut with an **ultramicrotome** using specially polished diamond blades (see, e.g., Figs. 2.7, 2.92). Alternatively, living tissues can be cryo-fixed by rapidly cooling them to below −150°C. This causes the water in the cells to solidify without crystallizing. Then the frozen specimen is shattered open, and a replica is made by taking a vapor-deposit cast of the broken edge that can then be studied in the TEM (**freeze fracture**; see, e.g., Figs. 2.8, 2.26a, 2.84, 2.93a, c).

Recently, relatively thick slices were observed using acceleration voltages between 300 and 700 kV, and images of particular points on the specimens taken from precisely defined angles were stored digitally. A three-dimensional representation of the object is computed that, just like with the confocal laser scanning microscope, represents the spatial structure of the object.

The surface structure of nontransmitting objects can be observed using a **scanning electron microscope** (**SEM**). This process works much like a television. The specimen is scanned in rows using a fine beam of electrons. Each part of the specimen touched by the electron beam at a particular moment emits secondary and backscattered electrons. They arrive synchronously with the scan of the specimen surface and contribute to the row-by-row image construction on the monitor. Objective lenses are not involved. SEM images are distinguished by their high depth of focus and a particularly plastic representation of specimen sculpting (see, e.g., Figs. 3.3c, d, 3.10, 3.11, 3.14).

2.2 The Plant Cell

2.2.1 Overview

Figures 2.2, *2.7*, and *2.8* show typical plants cells as seen with light and electron microscopes. All the most important cell components are shown. These will be characterized using keyword-like definitions. Structure, function, and genesis of the individual organelles will be treated in detail in the following sections. An overview of the ultrastructure is shown in *Fig. 2.6*.

Organelles (Latin *organellum*, little machine). Organelles are subcellular functional units.

Cell Wall. This surrounds the living cell body (protoplast) as a shape-defining exoskeleton and contains strong (rip-proof) fibrils of cellulose or chitin, filled with fine canals (**plasmodesmata**) (Greek *désmos*, bond) – plasma bonds between neighboring cells.

Cell Membrane (**plasma membrane**). This is a membrane (Latin *membrána*, skin) that surrounds the entire protoplast; like most membranes, it is selectively permeable: it allows water and uncharged molecules through, but ions and larger polarized particles are only allowed through when the specific translocators are present in the membrane.

Membranes. These are 6–11 nm thick, viscous or stringy; the lipid bilayer is a fundamental component of all membranes. It is pierced by integral membrane proteins. Peripheral membrane proteins adhere to the surface. Membranes delimit compartments, surrounding them without a break – they have no sides; they divide inside from outside.

Cytoplasm (Greek *kýtos*, bubble, cell, and *plásma* structure). This is a viscose or jellylike mass of the cell in which the various organelles lie. Many metabolic reactions take place here. During cell fractionation, it separates out as **cytosol** (soluble fraction).

Cytoskeleton. Endoskeleton is able to locally support the cytoplasm (sol → gel). It also enables transport processes within the cell with the help of motor proteins (e.g., plasma streaming, vesicular transfer, chromosomal changes during nuclear division), in plants mainly microtubules (Latin *túbulus*, tube) and actin microfilaments (Latin *filum*, thread). Plant cells also contain an additional cytoskeleton element, the **FtsZ protein** (see Sect. 2.3.1), whose function was first described for plastid division. The gene of this cytoskeleton protein, first identified in bacteria, is found in the nuclear DNA of plant cells.

Ribosomes (Greek *sóma*, body, particles). These are small (30 nm) dense particles in the cytoplasm and on endoplasmic reticulum (ER) membranes. They are mostly clustered into **polysomes**. They are ribonucleoprotein complexes and are the site of protein biosynthesis (translation).

Endoplasmic Reticulum (**ER**) (Latin *reticulum*, network). This occurs throughout the cytoplasm as a branched membrane system: **rough ER** membranes have polysomes scattered on the outside and **smooth ER** membranes are without polysomes. The inner space of the rough ER is generally flat. The ER membranes run parallel through the cytoplasm (double membranes); they are called **cisternae**. As a typical example, the **nuclear envelope** (perinuclear cisternae) is formed of ER cisternae.

Dictyosomes (Greek *díktyon*, net). These are small stacks of ribosome-free cisternae (Golgi cisternae) that receive streams of small vesicle material from the rough ER, convert it to secretions (e.g., proteins, cell wall material), and deliver them to the cell membrane via the **Golgi vesicles.** The secretions are transported to the outside from there (**exocytosis**). All the dictyosomes of a cell are collectively called the **Golgi apparatus**, after C. Golgi (pronounced Góldschi), the discoverer of the organelle.

Vesicle (Latin *vesíca*, bubble; diminutive *vesícula*). These are small round compartments. They are frequently used for the displacement of substances within the cell. They arise by fission of larger compartments. Vesicles of

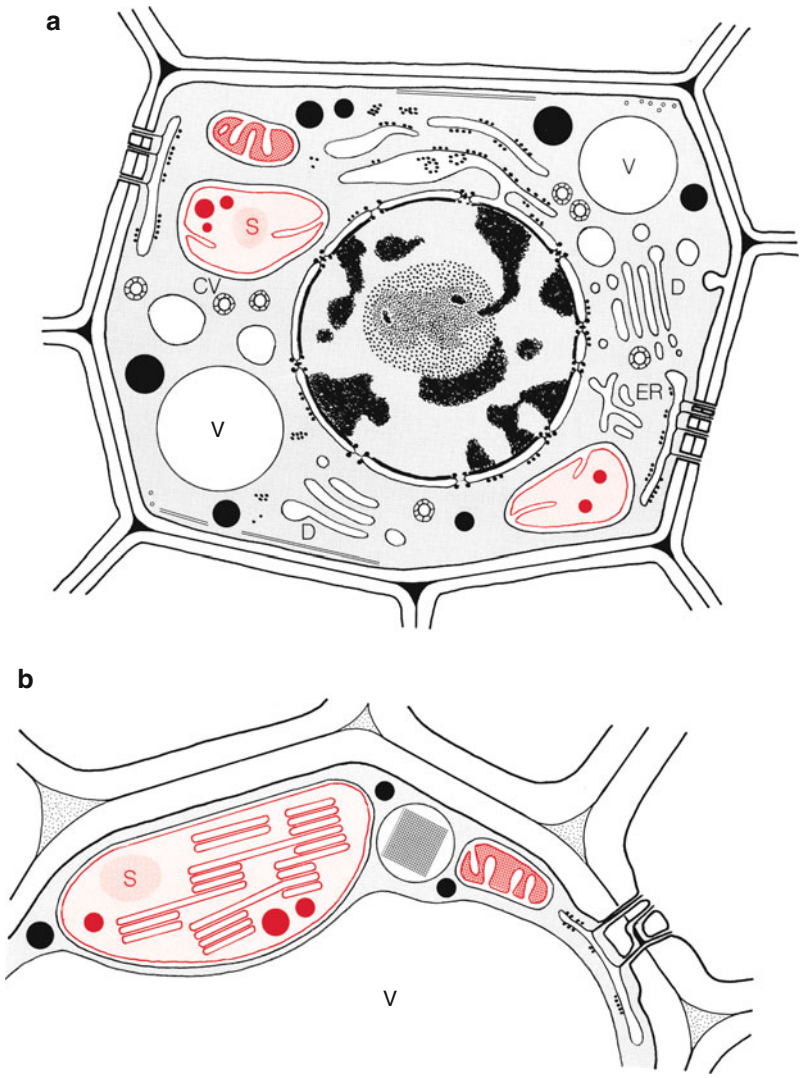

Fig. 2.6

Ultrastructure of plant cells. (a) Embryonic cell: cell wall with middle lamella and plasmodesmata; two dictyosomes in the cytoplasm, smooth and rough endoplasmic reticula, ribosomes and polysomes, various vesicles (including coated vesicles), and lipid droplets (oleosomes, *black*). Below the cell membrane, at places there are microtubules, both transverse and longitudinal; vacuoles; a nucleolus and dense chromatin in the central cell nucleus; two proplastids (*light red*, with plastoglobuli and starch); and a mitochondrion (*dark red*, with cristae). The organelles in *red* have their own DNA; nonplasmatic compartments are in *white*. (b) Tissue cell section of a leaf cell with a greatly enlarged vacuole. Mature primary wall (saccoderm), intercellular spaces (*dotted*) in the cell corners; in the cytoplasm, there is a mitochondrion, rough endoplasmic reticulum and oleosomes, a peroxisome with catalase crystals, and a chloroplast with thylakoids, plastoglobuli, and starch grains. *CV* coated vesicles, *D* dictyosomes, *ER* endoplasmic reticulum, *S* starch, *V* vacuole

only 0.1 µm diameter and with a dense protein coat (**coated vesicles**) are a special form. Transport by vesicle streams is collectively referred to as **membrane traffic** (**cytoses**).

Vacuoles (Latin *vácuus*, empty). These are large round compartments. They form the **central vacuole** in mature plants, which often makes up more than 90% of the cell's volume. They usually contain the often acidic **cell fluid**

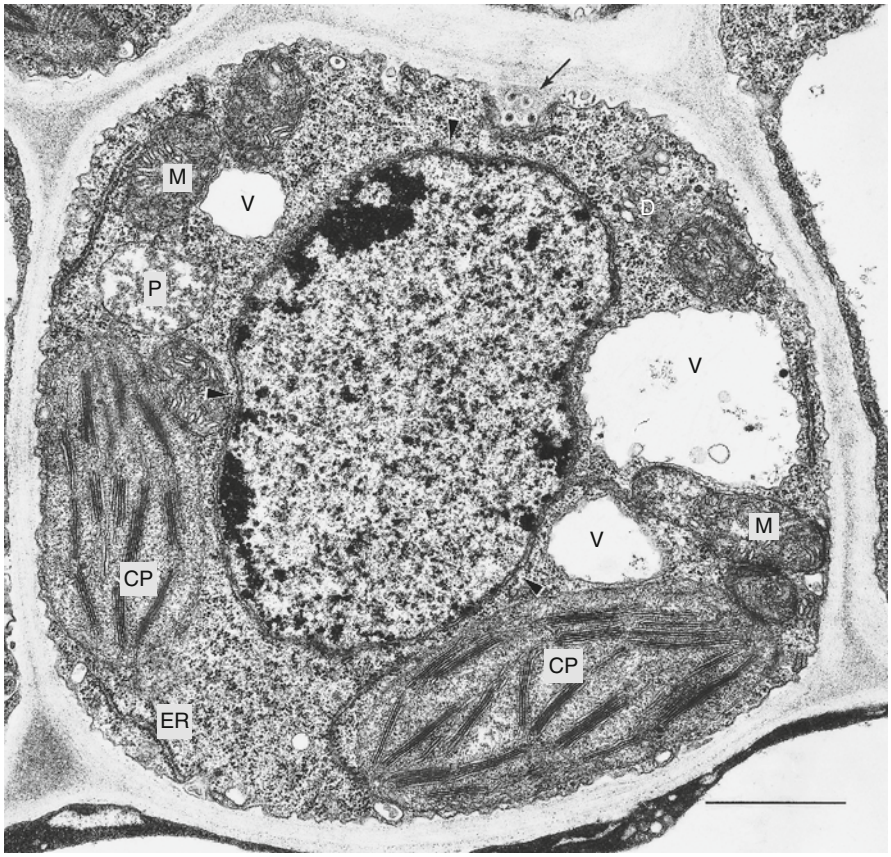

Fig. 2.7

Transverse section of a plant cell in an EM (ultrathin section, phloem parenchyma cell of the green bean *Phaseolus vulgaris*). This glandular cell shows many features of younger, more metabolically active cells (several small vacuoles, cytoplasm full of ribosomes and polysomes, but it also contains chloroplasts, mitochondria, and peroxisomes). The nucleolus is outside the plane of the section. *Arrowheads* nuclear pores, *arrow* plasmodesmata. There are four coated vesicles near the dictyosome. The central cell nucleus is dominated by diffuse euchromatin, and at places there are thicker patches of heterochromatin. *Scale bar* 1 μm. *CP* chloroplasts, *M* mitochondria, *P* peroxisome; other labels as for Fig. 2.6 (Specimen and EM image from H. Falk)

(cell sap) that is separated from the cytoplasm by the **tonoplast** (vacuolar membrane) (Greek *tónos*, tension, pressure, and *plásis* creation). They frequently contain storage and waste products; they also contain pigments and other secondary plant substances, including toxins.

Peroxisomes. These are relatively large, dense vesicles (about 1 μm diameter) that can contain, among other enzymes, the enzyme catalase, which breaks down the hydrogen peroxide (H_2O_2) generated by metabolic processes.

Oleosomes (Latin *óleum*, oil). These are oil droplets in the cytoplasm; earlier they were termed "spherosomes" because of their round shape.

Plastids. All green cells of algae, mosses, and vascular plants contain the chlorophyll-containing **chloroplasts** (Greek *chlorós*, yellow-green), the photosynthetic (Greek *phos*, sunlight) organelles. The conversion of light energy into chemical energy proceeds according to a complex membrane system formed of chlorophyll-bearing membrane cisternae (**thylakoids**; Greek *thýlakos*, sack). This is where adenosine triphosphate (**ATP**) is formed (chemical energy is generated by cleaving the terminal third phosphate residue and can be used for energy-requiring reactions – synthesis, motion, active transport across membranes, etc.; see Sect. 5.1.5). There are other types of plastids in the cells of nongreen tissues in the organisms previously

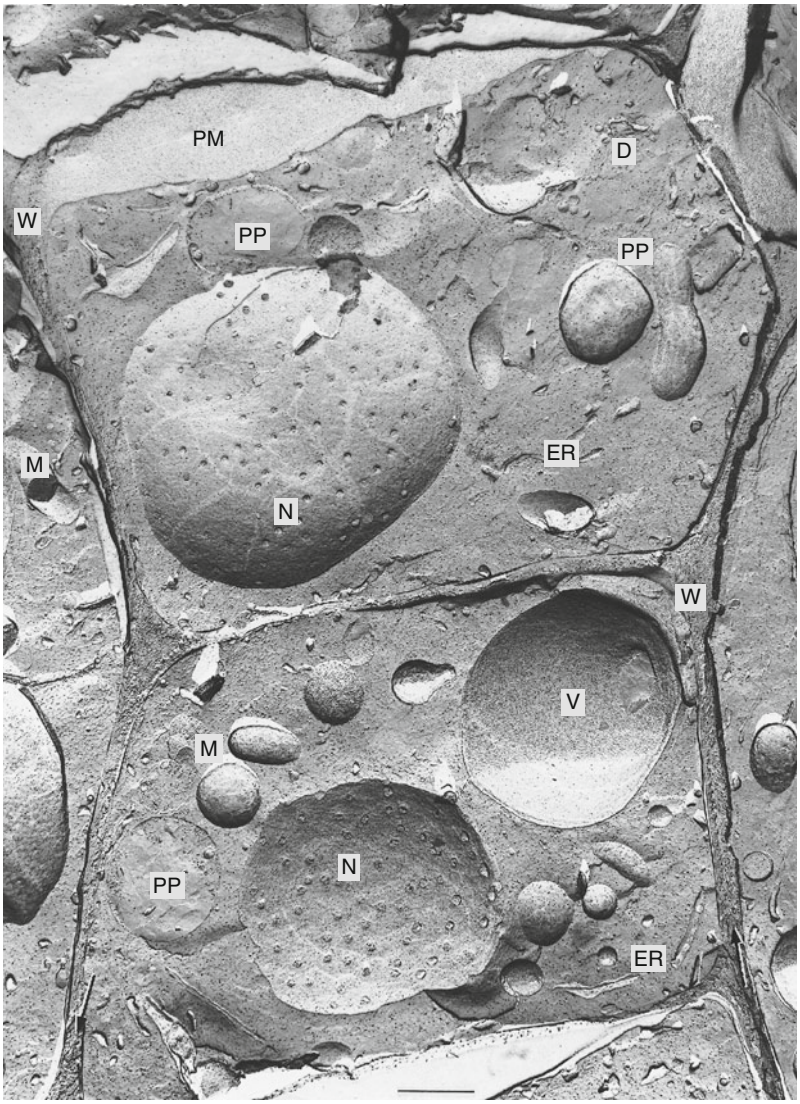

Fig. 2.8

Embryonic plant cell from a cauliflower stem bud in an EM (freeze-fracture specimen). The shattering of cryogenically fixed cells occurs in part along membranes that run parallel to the surface of the fracture. This is the case in the sheath membranes of the two-cell nuclei showing numerous nuclear pores. Mitochondria and proplastids are in part fractured and in part visible in surface relief. Also cell membranes (plasma membranes) and tonoplast membranes of vacuoles are partly seen in section, in other places, they are visible in surface view. Cisternae of the endoplasmic reticulum and a dictyosome are also visible. Cellulose fibrils are partially visible in the cell wall (*arrow*). *Scale bar* 1 μm. *ER* endoplasmic reticulum, *D* dictyosome, *M* mitochondria, *N* cell nuclei, *PM* plasma membrane, *PP* proplastids, *V* vacuoles, *W* cell wall (Specimen and EM image from K.A. Platt-Aloia and W.W. Thomson, with kind permission of *Journal of Electron Microscopy Technique*, John Wiley & Sons, New York)

mentioned (e.g., meristems contain small, nonpigmented **proplastids**). Yellow to red pigmented **chromoplasts** (Greek *chróma*, color) are used to attract pollinators. Plastids are always surrounded by a double membrane and contain their own DNA (ptDNA) and ribosomes that are different from those in the cytoplasm (plastid ribosomes). Replication is by division. All plastid forms are able to produce **starch grains** and **plastoglobuli** (oil droplets).

Mitochondria (Greek *mítos*, thread, and *chóndros*, kernel, because of the filamentous or short-oval outline). Like plastids, these have their own DNA and ribosomes (mitochondrial DNA, mtDNA, mitochondrial ribosomes). They formed from like by division. They are surrounded by a double membrane. They are organelles of **cell respiration** generating ATP. The formation of ATP and cell respiration components occurs on the inner coat membrane, whose surface is enlarged, owing to folding into the organelle body (**cristae**; Latin *crísta*, comb).

Cell nucleus (Latin *nucleus*, nucleus), **karyon** (Greek *káryon*, kernel). In most eukaryotic cells, it is the largest plasma organelle (usually about 10% of the plasma volume), generally present singly. It is surrounded by a characteristic double membrane with **nuclear pores;** there are no membranes inside, and it contains most of the cell's hereditary information: **genetic information** is stored in the base sequences of long **DNA** double helices. DNA molecules are central structural and functional elements of the chromosomes. In **chromatin**, the DNA is complexed with basic proteins (histones), and to a variable degree with non-histone proteins. The cell nucleus contains one or more nucleoli that produce the early stages of the cytoplasmic ribosomes. Reproduction occurs by cell division (**mitosis**): normally the nuclear envelope and the nucleoli degenerate, and the physiologically active noncondensed "working form" of chromatin condenses into the "transport form" of individual chromosomes. The term chromosome has originally been developed to describe the rod-shaped or threadlike, readily stainable structures chromatin forms during mitosis. Chromosomes are distributed evenly among the daughter cells by the mitotic spindle (a cytoskeleton structure mainly made of microtubules). The novel formation of the nuclear envelope and nucleoli and the decondensation of the "euchromatic" parts of the chromatin take place in the daughter cells. Heterochromatin condenses and remains inactive. The synthesis of RNA happens on particular DNA sequences (active gene transcription). The cell cycle is composed of successive regularly repeated stages. In the S phase of the cell cycle, the DNA is replicated and the chromosomes are doubled up.

Differentiation processes change the cells, but many organelles retain their form and function. Generally, only plastids, vacuoles, and cell walls undergo more changes.

2.2.2 The Cytoplasm

The **cell sap** or **hyaloplasm**, the fundamental part of the cytoplasm, is the thick fluid or jellylike mass in which the ribosomes and cytoskeletal elements lie and in which the plastids, mitochondria, cell nuclei, and often also aggregates of cell reserve substances (oleosomes, in fungi the glycogen granules) are embedded. It is rich in enzyme proteins. The total concentration of protein is between 10% and 30%. A significant portion of the water is bound with proteins. Active, ATP-driven ion pumps in the neighboring membranes maintain a special **ionic environment** in the cytoplasm. The cytoplasm is rich in K^+ and poor in Na^+, and it contains very little Ca^{2+} compared with the outside environment. The pH is around 7. This is an optimal pH for the enzymes in the cytoplasm.

Many important **reactions and metabolic pathways** (cycles) take place in the cytoplasm (glycolysis, the formation of storage lipids, the synthesis of amino acids, and, on the ribosomes, protein biosynthesis and also the synthesis of nucleotides and saccharose; see Sects. 5.9–5.16). Pharmacologically important substances (alkaloids, glycosides) are also manufactured in the cytoplasm of many plant and fungal cells and are shifted to the vacuoles or cell walls, where they are stored. Fatty acid synthesis also takes place in the cytoplasm of fungi and animals, a process that in plants occurs in the plastids.

The cytoplasm can vary in viscosity. The cytoskeletal elements exist to strengthen and stiffen the cell structure. Solutions of globular macromolecules (spherocolloids, to which the enzyme proteins of the hyaloplasm belong) retain a low viscosity even at high concentrations. Linear colloids (long-chain particles) become gelatinous at quite low concentrations. Their relatively large surface areas mean they tend to mat together. Actin filaments and microtubules are exceptional linear colloids. They are also polymers of globular proteins (generally, this term is used even though, in this case, the chain is a linear aggregation rather than having the more usual covalent bonds one would expect in a polymer). In the living cell, these polymers can be quickly broken down and rebuilt again, enabling the viscosity of the cytoplasm to be rapidly adapted according to need. As plant and fungal cells posses a solid exoskeleton in their cell walls, liquid cytoplasm is more commonly seen than in animal cells or in the naked cells of many flagellates and lower fungi. Preferentially, the outlying plasma zone, the **ectoplasm** (cortical plasma; Latin *cortex*, bark) is often present as a gel, whereas the inner **endoplasm** is often liquid. Distinct plasma streaming is restricted to the endoplasm.

Rapid **plasma streaming** can be seen in especially large cells. It serves for rapid intracellular substance transport, where

diffusion alone would not be sufficient. Two sorts of streaming have been recognized: **rotational** and **circulational streaming**. Plasma rotation is where the endoplasm constantly circulates the central vacuole in loops or figures of eight. This type of plasma streaming is seen in the extraordinarily large internodal cells of *Chara* and *Nitella* (Fig. 10.124) but also, e.g., in the leaf cells of the well-known aquarium plants *Elodea* and *Vallisneria*. Cells that have apical growth (fungal hyphae, root hairs, pollen tubes), hair cells (e.g., stinging hairs of the stinging nettle), and many epidermis cells have plasma streaming that occurs in numerous, sometimes even counterrotational streams, or in plasma strands that span the central vacuole (*Fig. 2.58c*; for the physiology of intracellular motion, see Sect. 7.2.2).

The amoeboid motion of wall-less cells or plasmodia is also based on plasma streaming. The speed record of 1 mm s^{-1} is held by the shuttle cytoplasmic streaming in slime molds (*Fig. 2.9*). It is generated by hydraulic pressure streams, causing the contractile ectoplasm tube of the plasmodium to contract at some points and expand at others. The stream direction switches every 2.5 min. Not only in this case, but also in streaming processes that are magnitudes slower, the streaming is powered by the actomyosin system (see Sect. 2.2.2.2). However, in contrast to the shuttle cytoplasmic streaming seen in myxomycetes, it is not hydraulic but is generated by shearing forces that shift the endoplasm relative to the stationary ectoplasm.

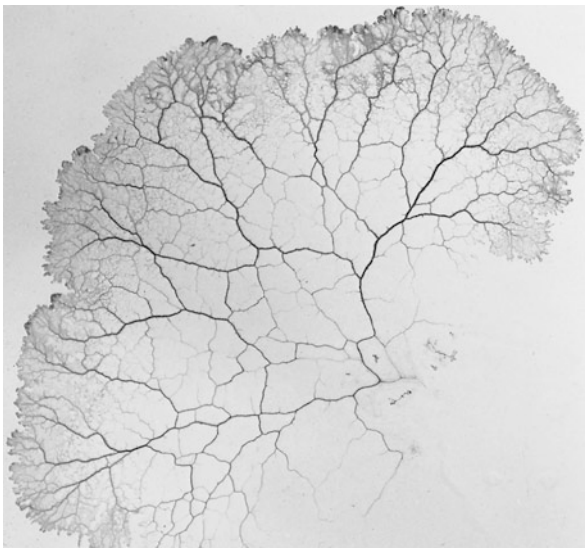

Fig. 2.9
Plasmodium of the slime mold *Physarum confertum* (×1.25) (Image from R. Stiemerling)

2.2.2.1 The Cytoskeleton

Gymnoblasts (cells without walls) tend to reduce their surface area by rounding. This is caused by boundary surface forces. Artificially generated wall-less cells of plants, fungi, and bacteria also take on a spherical form (see *Fig. 2.48*). Deviations from the spherical form are possible when there are stiffening structures outside the cell membrane (**dermatoblasts**, with a cell wall) and/or with a **cytoskeleton** in the cytoplasm itself. The cytoskeleton is especially well developed in gymnoblasts, as found in the wall-less unicellular organisms as well as in the tissue cells of animals and humans. The cytoskeleton protein actin makes up about 15% of the total protein content of the amoeboid multinucleate plasmodia of slime molds (*Fig. 2.9*). Whereas cell walls normally only change slowly through irreversible growth and are only rarely broken up again, the cytoskeleton can be rapidly broken down and rebuilt. It has proven to be a **dynamic construction** that defines the form and architecture of the cell and also all cellular movements.

The cytoskeleton of algae, higher plants, and fungi primarily consists of actin microfilaments and microtubules (*Fig. 2.10*).

Actin was first isolated from muscle fibers. Later, its widespread dispersion throughout eukaryotic cells was shown. The globular actin molecule (**G-actin**) has a diameter of 40 nm and a mass of 42 kDa. An ATP binding site is located between the large C-terminal domain and the smaller N-terminal domain. Actin filaments are formed easily in solutions of G-actin (**microfilaments**; **F-actin**; *Fig. 2.11*). The ATP in G-actin is hydrolyzed (split), and ADP remains bonded to the F-actin protomers. ATP hydrolysis is not essential for filament formation but allosteric effects promote it by stabilizing the filaments.

Microfilaments show **kinetic polarity**: the addition of further actin molecules occurs mainly at the so-called plus (+) end. Filament formation in living cells preferentially starts at special nucleation sites occupied by actin-binding proteins (e.g., α-actinin). Surprisingly, the plus end of the filament sticks fast to this formation center. The growth of microfilaments thus occurs by elongation of the fixed end, not at the free end (microtubules behave the other way round in this aspect).

The speed and (dimension) magnitude of the growth of microfilaments, including also their position and orientation, can be affected by many natural and artificial factors. Actin-associated proteins can stabilize or destabilize, cross-link or bundle, arrest growth of, or split microfilaments in living cells. The effects of

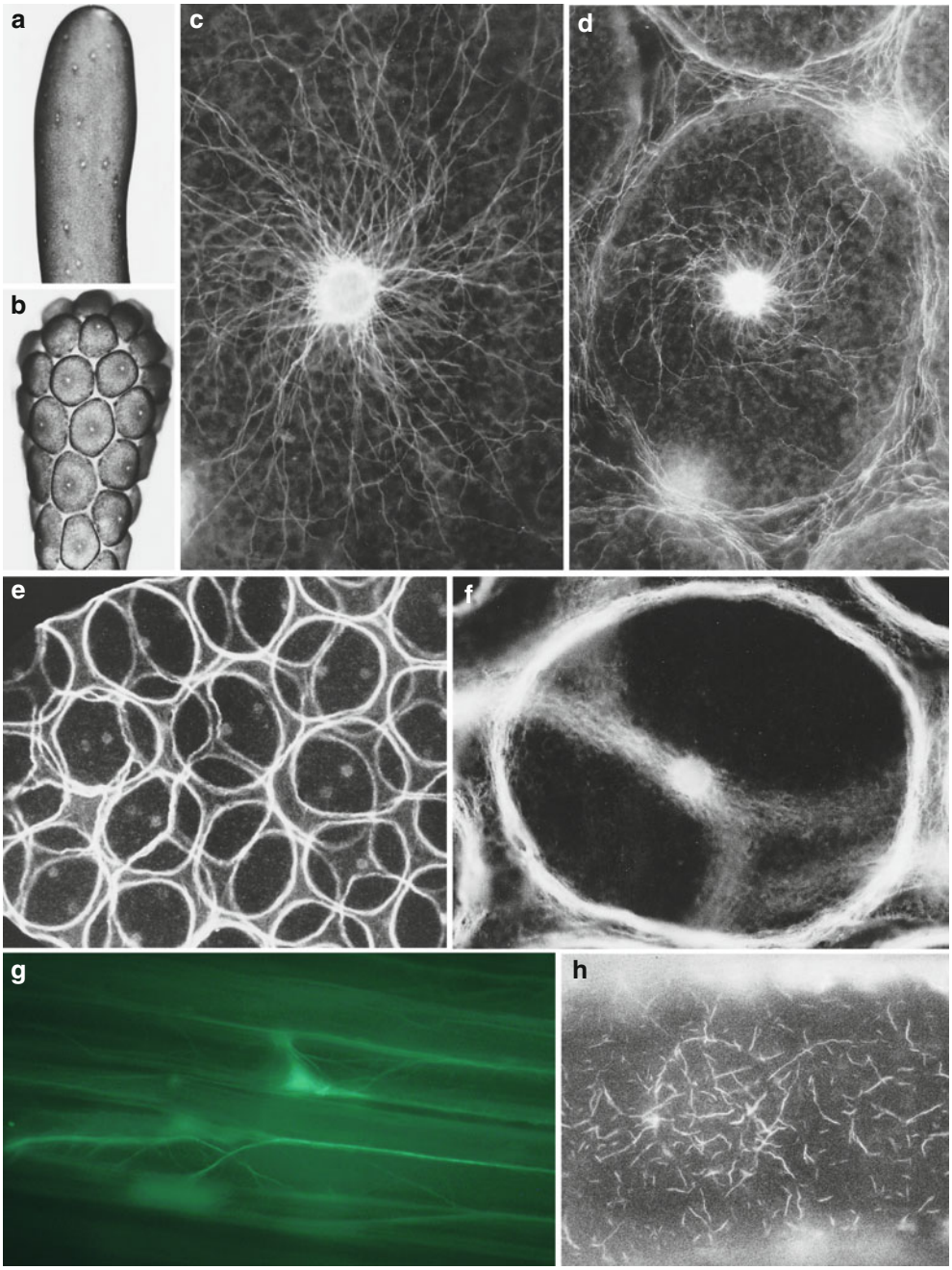

Fig. 2.10

Cytoskeletons in plant cells. (a–f) Cyst formation in the dasycladaceous *Acetabularia cliftoni* (see also Fig. 10.112): (a, b) migration of the secondary nucleus into an algal umbellate cell and the start of cyst formation by free cytogenesis (see Sect. 2.2.3.6; ×30); (c, d) microtubules made visible by indirect immunofluorescence; in the center of each picture is a nucleus (c ×350, d ×235); (e, f) localization of actin microfilaments during cyst formation; greater magnification (f) reveals individual filaments (e ×60, f ×235). (g) Actin microfilaments in living epidermis cells of *Arabidopsis thaliana* using fluorescence microscopy with indirect marking of an actin-binding protein (Kost et al. 1998) fused with green fluorescent protein (GFP) (×630). (h) Breaking down of the microfilaments in epidermis cells of an onion bulb using cytochalasin and fluorescence microscopy marked using phalloidine–rhodamine (×400) (Specimens and images from a–f D. Menzel and h H. Quader)

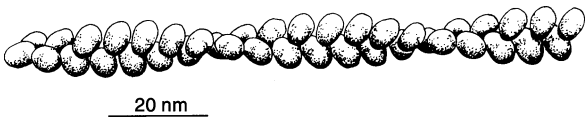

Fig. 2.11

Actin microfilament. The globular (more precisely, ellipsoid) actin monomers aggregate to form helices of about two molecules per twist. This gives the microfilament the shape of a tight double helix with a periodicity of about 40 nm

these proteins (in the well-researched mammalian cells, there are over 100 different types) are due to the highly dynamic actin cytoskeleton (Higaki et al. 2007). Two antagonistic proteins, **profilin** and **actin depolymerization factor** stand out in plants. They occur enriched everywhere where there are localized structural formations on cell surfaces, e.g., accelerated apical growth of cells (roots hairs, pollen tubes) and also in cell division (cell plate formation and primary plasmodesmata: see Sects. 2.2.3.6, 2.2.7.3). Under experimental conditions, **cytochalasin B**, a fungal toxin, causes microfilaments to be broken down. Intracellular processes that depend on microfilaments, such as cytoplasmic streaming or chloroplast movements, are blocked by cytochalasin. **Phalloidin** (a toxin extracted from the death cap, *Amanita phalloides*) has the same effect but causes all the cellular actin to aggregate into filaments that cannot be broken down (Ph-actin), thus suspending the living dynamics of the cytoskeleton. Actin is one of the most highly conserved eukaryotic proteins; its amino acid sequence has hardly changed at all during phylogeny. However, there are several actin genes in the genomes of most eukaryotes, each of which has slightly different products. They are termed isotypes or isovariants. The γ-isotype is generally expressed in nonmuscle cells.

The molecular building block of **microtubules** (*Fig. 2.12*) is a dimer unit of two similar but not identical proteins: α-tubulin and β-tubulin (heterodimer). **Tubulin** heterodimers (100 kDa) have a strong tendency to aggregate when in the presence of GTP and in the absence of calcium ions. The microtubule (Latin *túbulus*, small tube, tubule) is their typical "self-assembly" structure. Its wall is generally composed of 13 protofilaments (longitudinal series) of identically oriented tubule heterodimers. The outer diameter of the tubular quaternary structure is 25 nm, compared with the only 6 nm diameter of actin filaments. Microtubules form comparatively stiff lengths that snap under excessive bending forces, although such conditions normally do not occur in living cells.

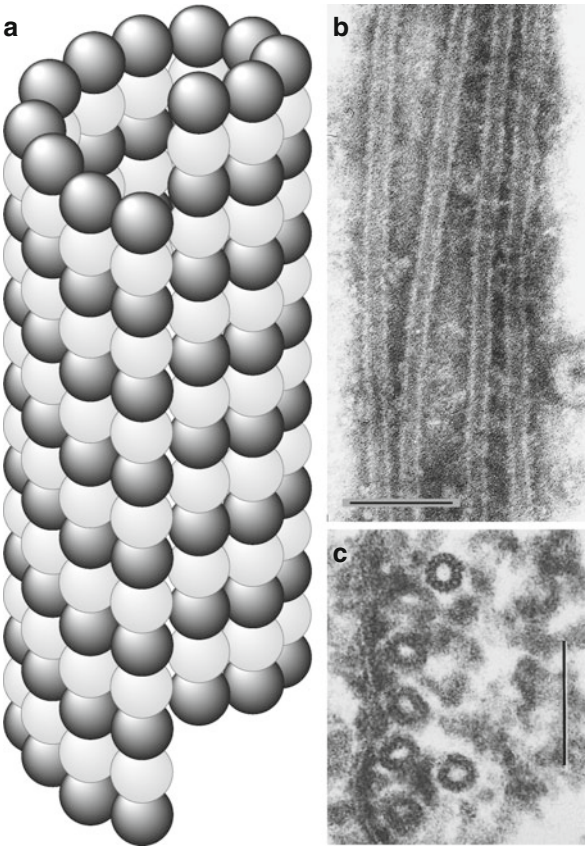

Fig. 2.12

Tubulin and microtubules. (a) Heterodimers of globular α-tubulin (*light*) and β-tubulin (*dark*) (each about 50 kDa, 4-nm diameter) are arranged in longitudinal rows – the protofilaments. Thirteen protofilaments form the hollow cylindrical microtubule. The heterodimers of neighboring protofilaments are lightly offset from each other, resulting in a flat helical structure. This so-called seam (at the front of the diagram) is not two α-tubulin units or two β-tubulin units against each other but α-tubulin next to β-tubulin. (b) Microtubules of the banana (*Musa paradisiacal*) in negative contrast. (c) Laterally sectioned microtubules of a preprophase band (*Fig. 2.13b*) in embryonic cells of the root cap of an onion; 13 protofilaments are partially visible. (b, c) *Scale bars* 0.1 μm (EM images from b I. Dorr and c H. Falk)

The molecular structures of α-tubulin and β-tubulin are very similar, although the amino acid sequences are only 40% similar. Surprisingly, they are homologous with the **FtsZ** cell division protein found in bacteria. Each tubulin molecule has a GTP/GDP binding site. Free tubulin heterodimers, which are commonly found in

abundance in most cells, are bonded with GTP and have GDP bonds after aggregation. This is analogous to the relationships of G-actin/F-actin with regard to ATP/ADP bonds.

Microtubule nucleation sites in the cells are called **microtubule organizing centers** (**MTOCs**). In particular, these are found in the basal bodies of flagella (centrioles; see Sect. 2.2.2.3), the two poles of the nuclear spindle (*Box 2.2*), as well as certain membrane regions. Like the microfilaments, each microtubule has a plus end and a minus end that characterize the regular orientation of the tubulin heterodimers alongside each microtubule. In contrast to the microfilaments, the minus end is fixed to the MTOC, and the plus end grows away from it. The short, left-handed helical nucleation sites producing new microtubules from the MTOCs are composed of several specific proteins and contain a third tubulin isotype, γ-tubulin. The minus end of the microtubules is the α-end and the plus end is the β-end. In higher plants, γ-tubulin is not only associated with microtubule nucleation sites, it is also more commonly found alongside the entire microtubule as well as on endomembranes (e.g., especially on the outer nuclear membrane).

Apart from the availability of tubulin heterodimers and GTP, the speed and magnitude of microtubule elongation depend on, and are controlled by, many factors. Thus, tubulin aggregation only occurs at calcium concentrations below 10^{-7} M. Various protein factors play an important role in living cells. They are grouped together as **microtubule-associated proteins** (**MAPs**). There are two classes of MAPs: the τ factor (55–65 kDa) that is incorporated into the microtubules, and high molecular mass MAPs (250–250 kDa) that usually occur as lateral 30-nm-long arms, standing out from the microtubules and acting as bonds between them and, e.g., membranes. Some of the high molecular mass MAPs are enzymes and are able to phosphorylate proteins, e.g., or are ATPases. The most important of these ATPases are dynein and kinesin (see the following section).

As for the microfilaments, the formation and breakdown of microtubules can be affected by specific drugs applied experimentally. The most historical of these is **colchicine**, an alkaloid from the autumn crocus (*Colchicum autumnale*). It binds to the β-tubulin of free tubulin heterodimers and blocks their integration into microtubules. These days, the stronger and more specific herbicides oryzalin and amiprophos methyl are used for the experimental breakdown of plant microtubules. **Taxol** (an alkaloid from yew, *Taxus baccata*) has the opposite effect. It stabilizes microtubules and reduces the pool of free heterodimers.

Microtubules in the same cell can often differ in stability, and one differentiates between stable and labile microtubules. Under the influence of colchicine, labile microtubules disaggregate (e.g., those of the nuclear spindle), but the stable microtubules found in flagella do not. Also, whereas flagellar microtubules are stable even at low temperatures and during fixation with osmium tetroxide, labile microtubules vanish in both cases. The wide distribution of labile microtubules was only demonstrated after glutaraldehyde fixation in electron microscopy was introduced. The variable stability of microtubules is possible due to the various tubulin isotypes (*Arabidopsis* has nine α-tubulins and six β-tubulins) and/or specific associated proteins.

Many cells have complex microtubule structures, sometimes only temporarily for time-limited functions, in other cases as permanent features.

The most well known example of a functional structure is the nuclear spindle (*Box 2.2*). However, the characteristic arrangement or relocation of microtubules is not restricted to mitosis but can also occur during other phases of the cell cycle in higher plant cells (**microtubule cycle**; *Fig. 2.13*). During interphase, the microtubules are mostly positioned immediately below the cell membrane in the cortical plasma. There they play an important role in the formation of the cell wall (orientation of cellulose microfibrils; local separation of the cell membrane from the cell wall for localized secondary wall formation like in the differentiation of helical vessels in xylem; see *Figs. 2.73c*, 3.24e) and in morphogenetic processes.

Prominent patterns of stable microtubules are widespread in cell-wall-less protists and spermatozoids, where they are associated with the reinforcement of characteristic cell forms and/or the anchoring of the flagellar apparatus.

Vertebrate cells have not only actin filaments and microtubules but also other cytoskeleton elements in the form of filaments whose diameter is about 10 nm. This places them between the 25 nm of the microtubules and the 6 nm of microfilaments; appropriately, they are called **intermediate filaments** (**10-nm filaments**). These can sometimes form dense, extended filament nets in mammalian cells. They are characterized by being insoluble, except in concentrated urea solutions. The intermediate filaments are bundles of long, straight protein molecules. To date, six subfamilies are known; the about 40 different intermediate filament proteins have homologous sequences (including the lamins of the nuclear lamina; see Sect. 2.2.3.4).

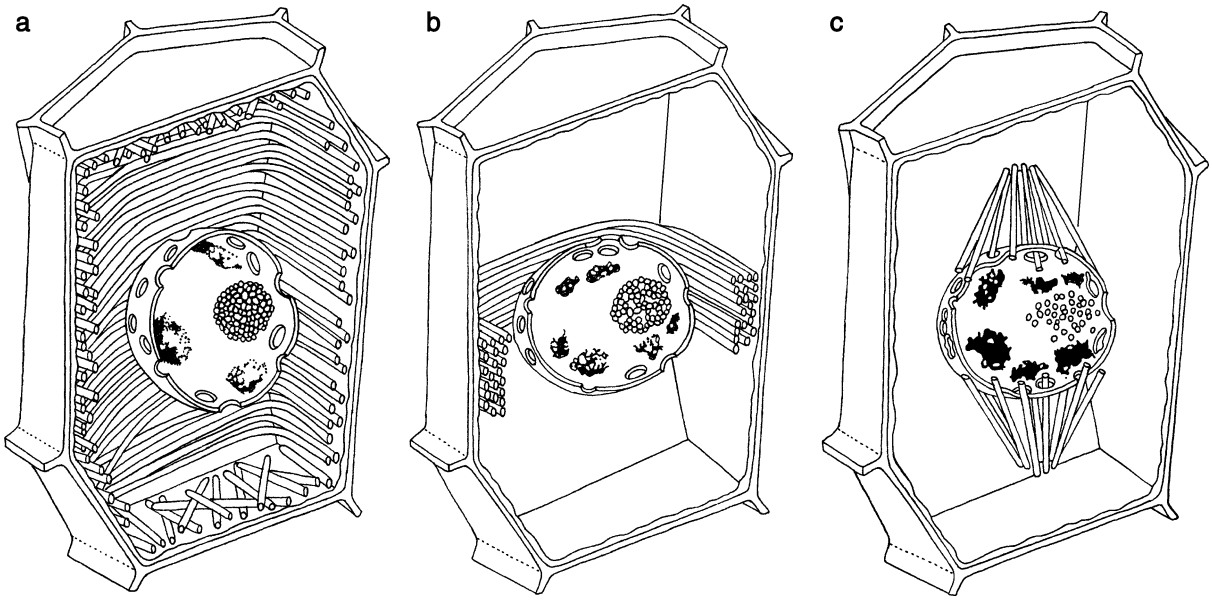

Fig. 2.13

Changes in the microtubule arrangement before the start of mitosis in the cells of the root meristem. (a) Interphase. (b) Formation of the preprophase band before prophase starts; its position marks the later spindle equator and the plane of cell division. (c) Late prophase (After M.C. Ledbetter)

2.2.2.2 Motor Proteins and Cellular Kinetic Processes

The cytoskeleton is significantly involved in the cellular kinetic processes (contraction, motility). On the one hand, it prescribes the direction of kinesis, like a track or road network. On the other hand, following Newton's principle whereby force and counterforce are numerically equivalent, every force-generating element is counterbalanced (compare muscles and skeletons). In the cell, specific ATPases function as chemomechanical energy converters (**motor proteins**, **motor molecules**) and convert the energy released by ATP hydrolysis into changes of conformation and thus into motion, and work directly together with the cytoskeletal elements. The two main cytoskeleton components each have a system that is widespread throughout the eukaryotes: the actomyosin system and the microtubule dynein/kinesin system.

Myosin is the partner of actin in the generation of thrust and shear forces in the ground plasma. Myosin (Greek *myon*, muscle) is a complex ATPase that is activated by actin. It has been extensively researched in the muscles of vertebrates and insects. The prolifically occurring myosin II has a quaternary structure (470 kDa) of two parallel straight "heavy" chains and four shorter "light" chains (*Fig. 2.14a, b*). The strongly anisometric particles have a long α-helical tail region and two N-terminal spherical head sections. These are identical to each other and operate independently of each other during contractile movements. ATPase activity and actin binding sites are localized; the Ca^{2+}-binding light chains lie adjacent. In transversely striped muscle fibers, myosin II aggregates above the tail domains to form distinctive stable myosin filaments. At great enough (about millimolar) Ca^{2+} concentrations, one of the two myosin heads lies up against an actin filament, and the products of the already hydrolyzed ATP are released. This results in a drastic conformation change in the myosin: the head folds down and shifts the microfilaments about 10 nm. Renewed uptake of ATP breaks the bond to the actin, and ATP hydrolysis causes the head to be raised again. In a muscle contraction, these processes repeat cyclically and actin and myosin shift against each other. The two-part system of actin and myosin filaments works not because the filaments themselves shorten, but because they shift. This sliding filament model is also basically true for the microtubule dynein/kinesin system.

Sequence comparison and motility assays (protein fractions are tested for their ability to trigger movement in isolated actin

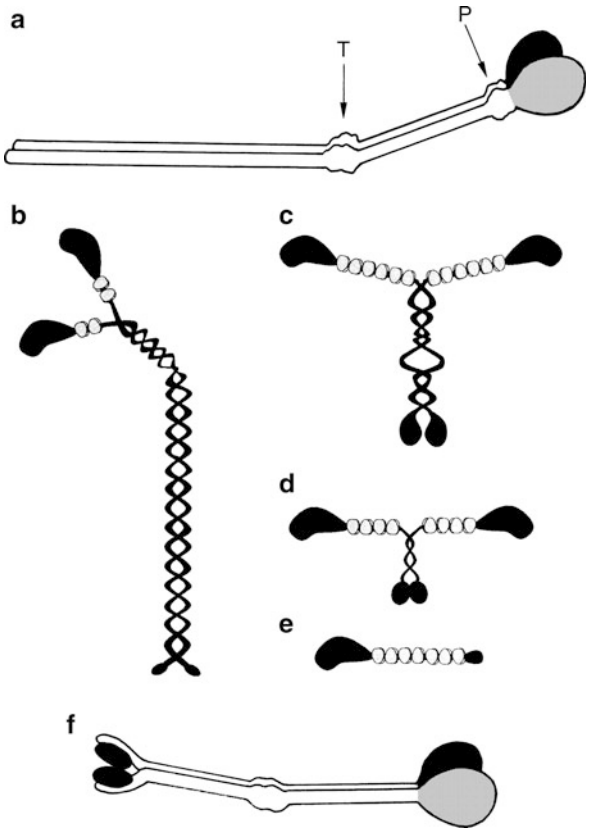

Fig. 2.14

Motor molecule. (a) Myosin II from mammalian muscles is the motor molecule that has been known for longest and is the best researched. It is divided by the proteinase trypsin (*T*) into head and tail parts. Papain (*P*) divides the head part into the N-terminal globular domains of the heavy myosin chains with actin-bonded sites and ATPase activity and a neck part with which the light chains are associated (not shown here). Neck and tail parts are stretched α-helical domains. The attack sites T and P lie in looser parts of the secondary structure at which the myosin particles are bent like joints. The opposing actin and myosin filaments shift as the joint is flexed at P. (b) Domain structure of myosin II. The globular units in the neck parts are the light chains (as in c–e). (c–e) Myosin from plant cells: (c, d) class XI and VIII myosins of sunflower and *Arabidopsis thaliana*; (e) class XII myosin from the siphonal green alga *Acetabularia*. (f) Kinesin; the two α-chains form globular domains at their amino ends (*right*) with ATPase activity and tubulin binding sites; two light chains are located at the C-termini. Class II myosins are 160-nm-long, and kinesin tetramers are only half as long (b–e After D. Menzel)

filaments) have identified numerous myosins that differ quite significantly from the myosin II found in muscle cells. This is especially true for plant myosins that are ordered into classes VIII, XI, and XII of the myosin superfamily (Fig. 2.14c–e). Their mode of action is similar to that of myosin II. However, their tail regions do not form myosin filaments (only found in muscle cells) but anchor themselves directly to membranes or vesicles or other cell structures (but not microtubules). Plasma streaming and chloroplast relocation are generally driven by the actomyosin system.

In contrast to the actomyosin system, microtubule-dependent movements use two different classes of motor proteins, dyneins (Greek *dýnamis*, strength, power) and kinesins (Greek *kínesis*, movement). **Dynein**, a high molecular mass complex, is especially prominent in flagella and cilia (see the following section). It is also generally found in nonflagellate cells as a simple cytoplasmic dynein. It works directly with **dynactin**, another large protein of the dynamin complex, and other associated proteins that change from case to case. The movements generated by dynein always occur in the direction of the minus end of the microtubule, functioning as a counterforce or track: dyneins are minus-end-directed motors (minus motors). In contrast, most **kinesins** are plus-end-directed motors (only a few structurally deviant representatives of the kinesin superfamily whose motor domain sits on the C-terminus instead of the N-terminus are minus motors). They were first discovered in the axial processes of nerve cells. Since then their occurrence in plants has been well documented. Their molecular form (Fig. 2.14f) is similar to that of myosins, which are also plus motors (but on actin microfilaments). Although kinesins are not related to myosins in terms of their sequences, the three-dimensional structures of the motor domains are similar in both cases.

Cellular movements sometimes happen without the systems described. In this way, the elongation or shortening of microfilaments or microtubules can cause organelle relocation or changes in conformation. The following describes a completely different mechanism, based on quite different molecules: the stalk of unicellular *Vorticella*s (sessile bell-shaped ciliates) contracts violently when the cell body is touched. A central strand in the stalk, the myoneme, twists up to form a flattened spiral (Fig. 2.15). The myoneme is mostly made up of a low molecular mass phosphoprotein (about 20 kDa) that belongs to the centrin (spasmin, caltractin) family. Its conformation, and thus also the form of the myoneme, changes

dramatically when calcium ions are bonded. ATP is not hydrolyzed in the process. However, it is necessary to have a comparatively slower process in order to remove Ca^{2+} and elongate the myoneme again after the contraction. The centrins are closely related to the calcium-binding protein calmodulin in terms of its amino acid sequence. With the aid of specific antibodies, centrin has been found in many eukaryotic cells and also in higher plants. It is principally associated with basal bodies and centrioles, as well as with anchorage structures of flagella (flagellate roots; see Sect. 2.2.2.3). It is also found in the centroplasm and in phragmoplasts during cell division (see Sect. 2.2.3.6). What function it plays here is as yet unknown.

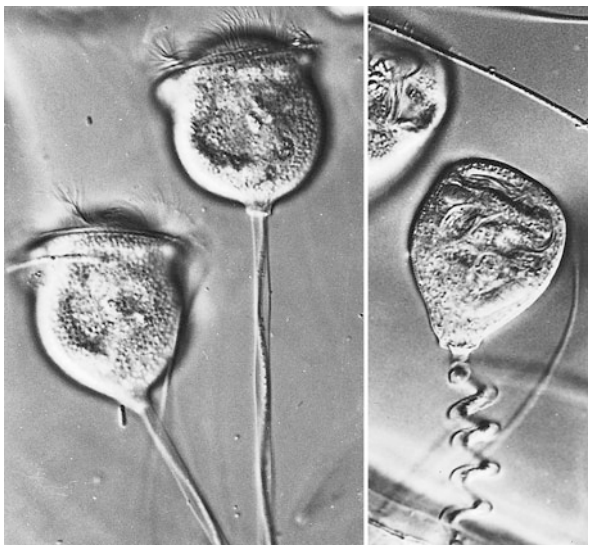

Fig. 2.15
The contractile spasmoneme of *Vorticella*: *left* in the elongated stalk, and *right* after stimulation, the spasmoneme and stalk are spirally shortened (×420) (Interference contrast image from P. Sitte)

2.2.2.3 Flagella and Centrioles

Wherever flagella occur in eukaryotes, their inner structure is basically the same. It is one of the most highly conserved structures.

Even the widespread **cilia** of animals and humans have basically the same ultrastructure. Cilia are shorter than flagella and always numerous on the cell in question (shimmer epithelial cells; unicellular organisms, ciliates). The analogous movement organelles, the **flagella** (Latin *flagellum*, whip, flagella) of bacteria have a completely different structure and function in quite a different way (see Sect. 2.3.2).

Transverse section of a flagellum shows a characteristic arrangement of 20 microtubules (*Fig. 2.16*). This is known as the **9+2 structure**. Two central single tubules (singlets) are symmetrically surrounded by a ring of nine

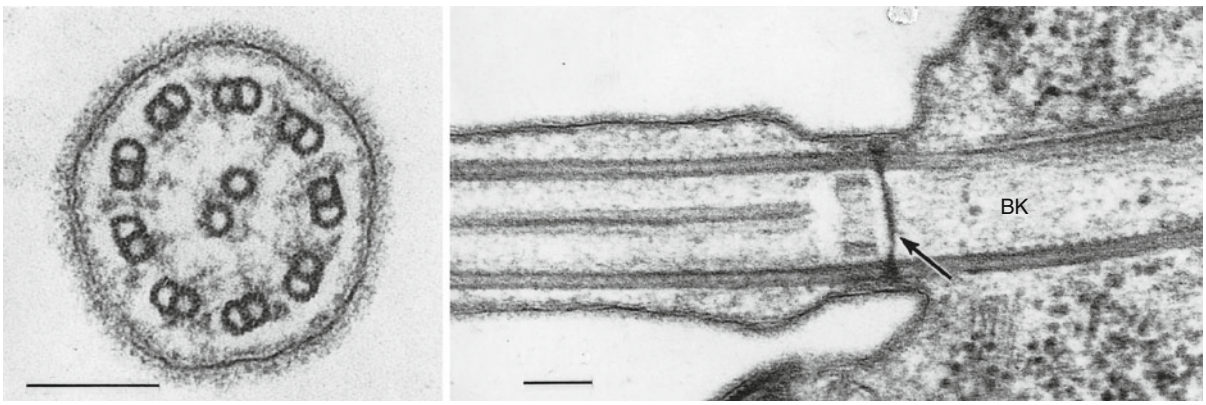

Fig. 2.16
Flagellum of *Scourfieldia caeca*, a green flagellate: *left* transverse section, *right* flagellum base with basal body lengthwise. The central singlets, which are lacking in the basal body, start 100 nm beyond this plate. In transverse section, the dynein arms and radial spokes can be interpreted. *Scale bars* 0.1 μm. *BK* basal body, *arrow* basal plate at the transition of the basal body to the flagellum (EM images from M. Melkonian.)

fused doublets (double tubules). The doublets are not exactly tangentially oriented; the so-called A-tubule is situated somewhat more toward the inner side than the B-tubule. Only the A-tubule is made up of 13 protofilaments. The B-tubule, which has a greater diameter, sits to the side of the A-tubule. It has 11 protofilaments and shares a further four protofilaments with the A-tubule, forming an entire (but not round) hollow cylinder. Together with numerous other proteins, singlets and doublets form the complex flagellar cytoskeleton (Fig. 2.17). Together with dynein as the motor molecule, they cause the whip motion. The entire motile structure, with a diameter of 200 nm, continues the whole length of the flagellum and is called the **axoneme** (Greek *áxon*, axis, and *néma*, thread).

The flagellum dynein is a very complex, tubulin-activated ATPase. The dynein of the outer arms has, e.g., a particle mass of nearly 2 MDa and consists of about 12 different protomers. Under the electron microscope, this shows up as a polynomial structure. The dynein arms that are exerted from the A-tubules and reach to the neighboring B-tubules can pull adjacent doublets past each other – the sliding filament model also holds true here. Radial spokes and nexin bridges convert the resulting lengthwise shift within the axoneme into the characteristic curving motion of the flagellum.

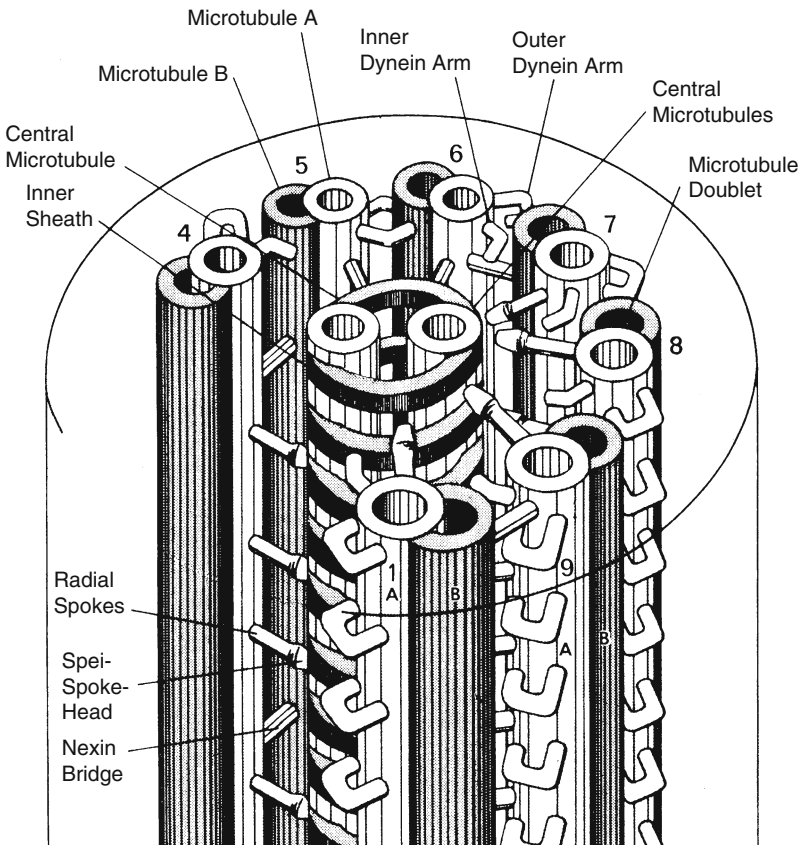

Fig. 2.17

The ultrastructure of a eukaryotic flagellum. The two central microtubules (singlets) are surrounded by a helical sheath that is bonded to the peripheral duplets by elastic radial spokes. Each microtubule A (*light*) in a duplet is loosely bonded by elastic protein arms (nexin) to the B microtubule *(dark*) of the neighboring duplet. Each A microtubule also has inner and outer dynein arms. The numbering of duplets starts in the plane of symmetry of the singlets with *1* and goes in the direction of the dynein arms (clockwise as seen from the flagellum base to the free end). For clarity, only seven duplets are drawn; the *hole* (duplets *2* and *3*) is shown by the interruption in the *circle*, the position of the plasma membrane is marked (After P. Satir)

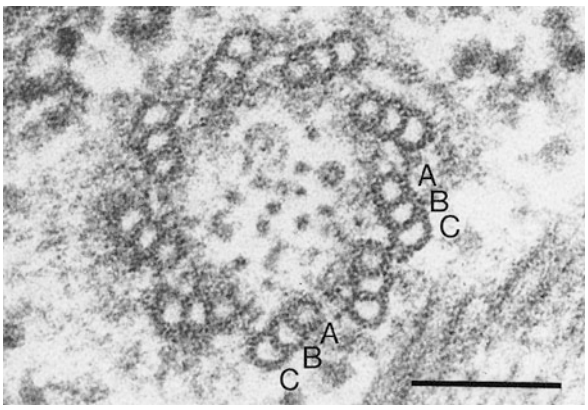

Fig. 2.18
Basal body of *Scourfieldia* in transverse section. In places, the protofilaments are visible in transverse section in the microtubule triplets. Only the innermost microtubules of the triplet (*A*) are complete; the two outwardly oblique microtubules (*B* and *C*) are furrowed, and they share some protofilaments with the adjacently in-lying microtubule. Microtubule C ends at the basal plate and microtubules A and B continue in the duplets of the flagellum axoneme. *Scale bar* 0.1 μm (EM image from M. Melkonian)

The surface of the flagellum is modified in many organisms. **Shimmer flagella** are densely coated with lateral filamentous **mastigonemes** (Greek *mástix*, flagellum), which greatly increases their friction in water (Figs. 10.21a–c, 10.98f). The mastigonemes are formed in the Golgi apparatus as a premolded secretion and reach the flagellum surface by directional exocytosis. **Whip flagella** are characterized by having an elongated, thin apical zone that only extends into the two singlet microtubules.

Each flagellum is anchored in the cortical cytoplasm with a **basal body**, a short cylinder of nine microtubule triplets (tubules A, B, and C); there are no central singlets (*Figs. 2.16*, *2.18*). The basal body is oriented perpendicular to the cell surface. During the formation of the flagellum, it functions as a developmental center from which the flagellum grows out. In the transition zone between the basal body and the flagellum shaft, the C-tubules end and the two singlets begin. A- and B-tubules of the basal body continue into the nine doublets of the axoneme. Thus, basal bodies also function as MTOCs, and the plus ends of the flagellar microtubules sit at the free end of the flagellum.

The structure of the basal body is identical to that of the **centrioles**. They generally occur in pairs. Where present, centriole pairs are usually (but not always) found in the poles of the nuclear spindle.

Basal bodies or centrioles do not arise from the division of self, but arise de novo every time. However, this often occurs in the vicinity of a basal body or centriole, which appears to have an inductive effect. The basal bodies of highly developed ferns and of gymnosperms that still form spermatozoids (in some cases with over 1,000 flagella per cell) arise in a spherical region of thickened plasma, the **blepharoplast** (Greek *blépharon*, lash; *Fig. 2.19*). Blepharoplasts represent a distinctly structured form of centroplasm (centrosome) that is normally structurally poorly defined, and which functions as an MTOC, organizing, e.g., the poles of the nuclear spindles in centrioleless flowering plants.

2.2.3 The Cell Nucleus

The genetic information of all cells – prokaryotic cells as well as eukaryotic cells – is digitally coded in the nucleotide sequences of the DNA molecules. In eukaryotic cells, the DNA can be found in the mitochondria and plastids, but is mainly found in the cell nucleus. It is the main compartment for the storage and replication of DNA, as well as for the synthesis (transcription) and processing of RNA. All these processes take place in the **nucleoplasm** (**karyoplasm**), which is separated from the surrounding cytoplasm by the double-layered **nuclear envelope**. It corresponds to the hollow spherical ER cisternae and characteristically has numerous **pore complexes** for the exchange of macromolecules between the nucleus and the cytoplasm.

Messenger RNAs (mRNAs), transfer RNAs (tRNAs), and the preribosomes formed in the nucleolus exit the nuclear space through these pore complexes, and nucleus-specific proteins gain entry to the nucleus through these pores (*Figs. 2.20*, 6.9, 6.17). There are no membranes in the nuclear space, but there are centimeter-long and even decimeter-long DNA molecules (1×10^{10} to 1×10^{11} Da). The essential functional and structural arrangement is maintained by the **nuclear matrix**, a sort of nuclear skeleton. The nuclear DNA–protein complex – the **chromatin** – is distributed throughout the gel of structural proteins. The basic histones play a dominant role as the proteins directly associated with the DNA. Chromatin occurs in various grades of condensation.

Replicative or transcriptive chromatin becomes more loosely structured **euchromatin** in the cell nucleus. Especially dense chromocenters are, comparatively, very much

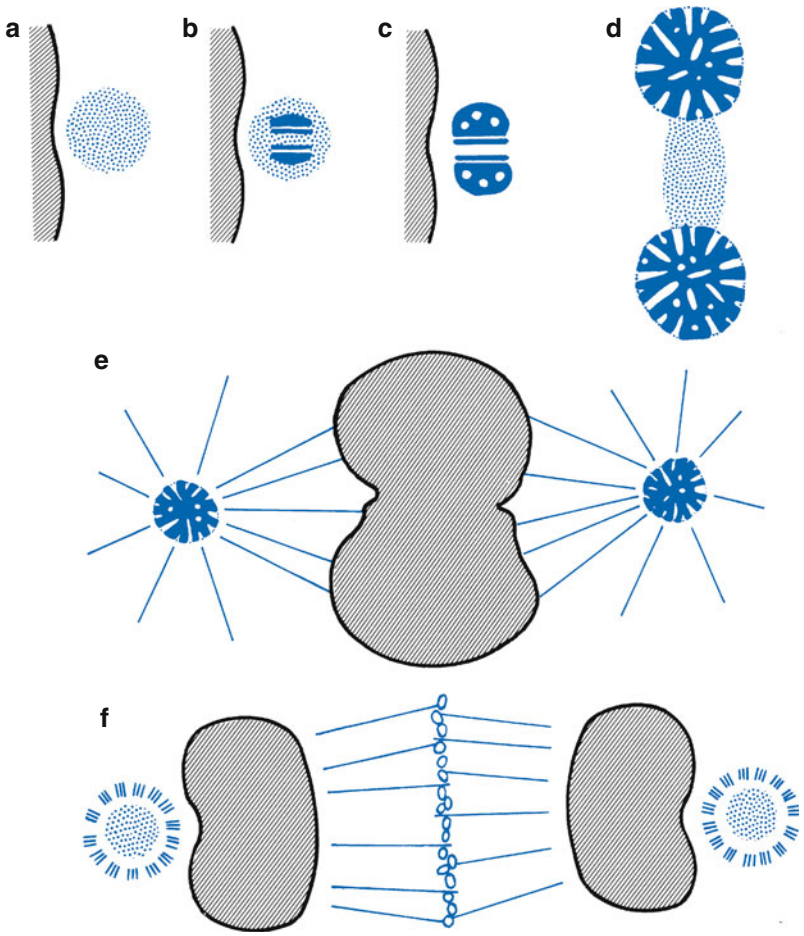

Fig. 2.19

Regeneration of centrioles/basal bodies during microsporogenesis of the water fern *Marsilea*. (a–c) A bisymmetrical structure forms in the dense plasma patches near the nuclear envelope from which two blepharoplasts (*colored*) arise. These separate before the next nuclear division (d) and have both spindle poles (e, f). Each blepharoplast produces about 150 basal bodies of the flagellate spermatozoids. The whole process shows that the characteristic complex structure of centrioles or basal bodies can arise de novo (After P.K. Hepler)

condensed and genetically inactive (**heterochromatin**). The same also applies to the compact **chromosomes** during nuclear division. **Gene activation**, as a prerequisite for transcription, happens locally in particular DNA sequences and comes about by various nonhistone proteins, especially by transcription factors. Different patterns of gene repression and gene activation determine the differentiation of tissue cells in multicellular organisms (**differential gene expression**).

Most of the molecular and supramolecular structural components of the cell nucleus are functional structures that are not permanent. The nuclear envelope and nucleoli decay, e.g., in the initial stages of nuclear division, are only built de novo in the end phase. The nuclear skeleton is also a dynamic structure whose molecular components change during the **cell cycle**, the characteristic sequence of stages between and during the nuclear and cellular divisions. The only nuclear component that normally – once formed by replication – does not change or break down is the DNA.

2.2.3.1 Chromatin

Most of the nuclear DNA is complexed with **histones**. Histones are commonly found throughout the eukaryotes. An exception is the dinoflagellates (Fig. 10.80),

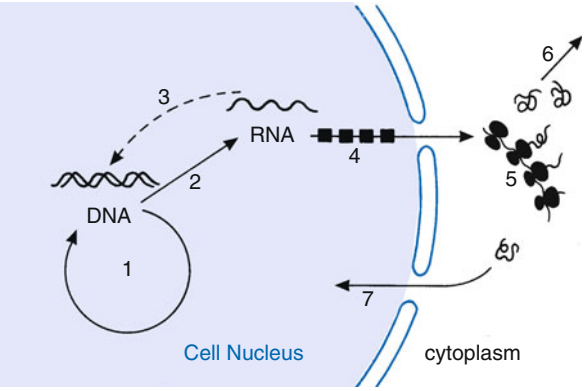

Fig. 2.20

The central dogma of molecular biology states that information flows in the cell from DNA via RNA to proteins: "DNA makes RNA makes protein." DNA serves not only as the template for RNA synthesis (transcription, *2*) but also contains instructions for its own replication (*1*). It is possible to reverse transcription (*3*, as used by RNA viruses that insert their genome into that of their host cell). In eukaryotic cells, these processes and the processing of newly formed RNA (*4*) take place within the pore complexes that permeate the nuclear envelope. The RNAs formed and processed in the nucleus become active in the cytoplasm during protein synthesis on ribosomes (translation, *5*). Many proteins drive the metabolism and energy exchange in the cell (*6*) as enzymes; others migrate into the nucleus (*7*), where they, e.g., participate in replication and transcription or assume important functions in the chromatin as DNA accessory proteins

Table 2.1

Overview of the five basic types of histone

Designation	Molecular mass (kDa)	Molecule shape
H1	>24	With two positively charged processes (C-terminus and N-terminus) and a globular central domain
H2A	~18.5	Globular N-terminal domains with increasing numbers of basic amino acid residues protruding laterally
H2B	~17	
H3	15.5	
H4	11.5	

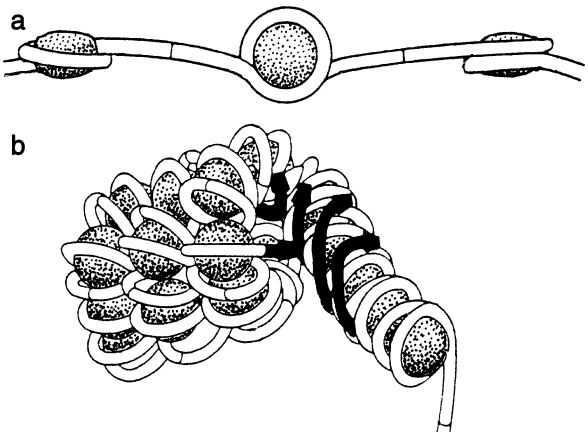

Fig. 2.21

Nucleosomes. (a) Beaded pattern: three histone octamers (*dotted*) surrounded by left-handed DNA double helices bonded by DNA linkers; *horizontal stripes* attack sites of *Micrococcus* nuclease. (b) Supranucleosomal structures, mediated by H1 (*black*); on the *right* nucleofilaments, on the *left* chromatin fibrils (H1 not is illustrated here) (After A. Worcel and C. Benyajati)

whose chromatin is organized differently. These unicellular organisms are typical eukaryotes in every other aspect. The mass ratio of histone to DNA is about 1:1. Histones only occur together with DNA in living cells. They are synthesized synchronously with the DNA in the replication phase of the cell cycle (S phase) in the cytoplasm and are immediately transferred to the cell nucleus. The strongly acidic DNA acts as a polyanion and attracts the histone molecule, which is basic as a result of its many lysine and arginine residues (pI about 12) and acts as a polycation (*Table 2.1*). The H1–H4 series is ordered according to the decreasing proportion of lysine and the increasing proportion of arginine. The histones, especially H3 and H4, have altered little during phylogenesis. There are also tissue-specific variants that in part deviate somewhat from each other in terms of activation of histogenes (isotypes) and in part differ in their posttranslational, reversible modification of the histone molecule (acetylation or phosphorylation of individual amino acids; see Sect. 6.2.2.2).

The four histones H2A–H4, which have similar molecular sizes and shapes, automatically form (even without DNA) flat-ellipsoid quaternary structures. In these particles, with diameters of up to 10 nm and thicknesses of 5 nm, two molecules of every histone type are involved – **histone octamers**, made up of **core**

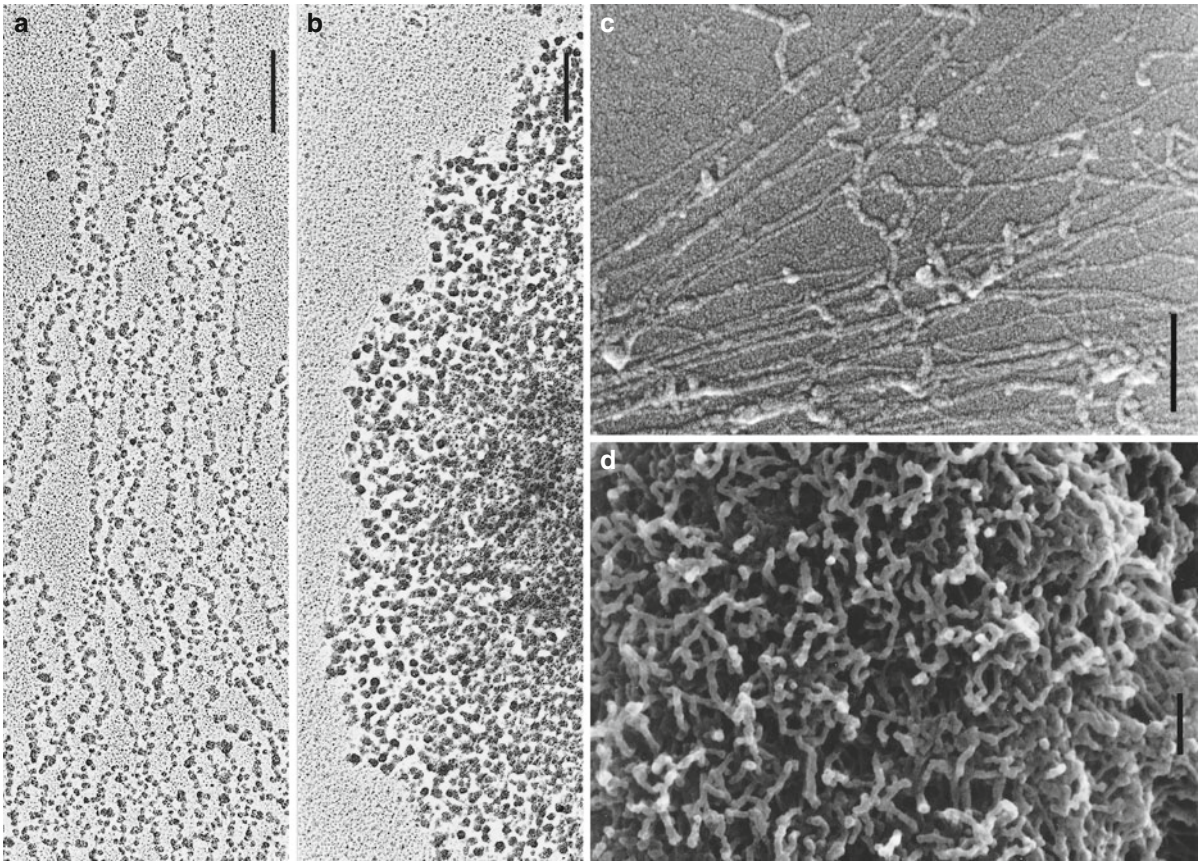

Fig. 2.22

Chromatin isolated from the nuclei of the onion *Allium cepa* (a, b) and barley *Hordeum vulgare* (c, d). (a) Beaded pattern of expanded chromatin under low ion concentration. (b) Supranucleosomal structures in physiological salt concentration (100 mM NaCl). (c) Chromatin after treatment with proteinase K; nucleofilaments and chromatin fibrils are visible next to naked DNA. (d) After a short proteinase K treatment, one chromosome develops lots of chromatin fibrils. *Scale bars* 0.2 μm (a, b EM images from H. Zentgraf; c, d scanning EM, SEM, images from G. Wanner)

histones. The edge of the histone octamer is flatly encircled by a 145-bp-long sequence of DNA, marking the location of the particularly basic N-termini of the histone molecule (*Fig. 2.21*). The DNA double helix twists just about twice around the histone octamer before going on to the next one. The approximately 60-bp intermediate linker represents the preferred attack site for endonucleases. Thus, cleavage events attack uniform nucleohistone complexes of the same particle mass, the **nucleosomes**. Electron microscope observations show greatly loosened H1-free chromatin with a typical beaded pattern (*Fig. 2.22a*).

This changes when H1 is added. This molecularly heavier histone (also less conserved in evolutionary terms) is not involved in the construction of the histone octamers or nucleosomes but preferentially binds, using nonspecific sequences, to linker DNA and densely binds nucleosomes to histone octamers already occupied by DNA. This gives H1 its other name of **linker histone**. It causes the chromatin to condense; increasing amount of H1 makes the molecule even more compact (*Fig. 2.22b–d*). Initially, **nucleofilaments** (elementary or fundamental fibrils) form with diameters of 10 nm; further condensation results in superstructures, e.g., **solenoids** (helix structures with six nucleosomes per turn; Greek *solén*, tube) or less regular zigzag structures, or supranucleosomal granula (**nucleomeres**). Finally, the **chromatin fibril** is formed, a 35-nm-thick filamentous structure. The DNA double helix packed into the chromatin fibril would be more than 20 times as long were it to be in its uncondensed form.

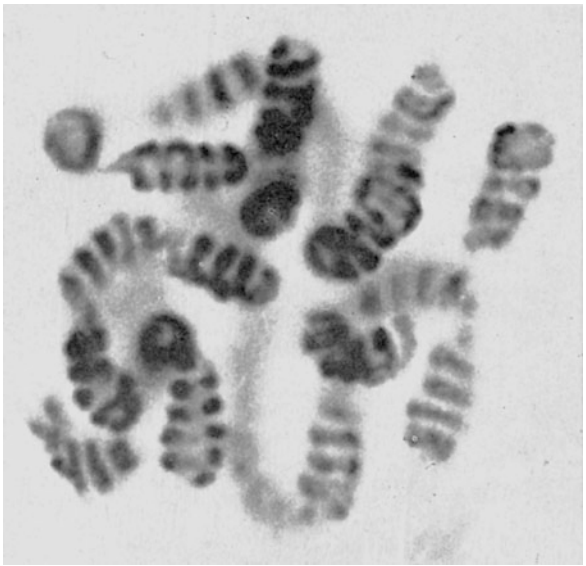

Fig. 2.23
Helical structure of meiotic chromosomes in *Tradescantia virginiana* (×4,050) (After C.D. Darlington and L.F. La Cour)

Even higher grades of chromatin condensation occur, particularly during nuclear division. Various nonhistone proteins then form a filamentous **chromosome skeleton** from which the chromatin fibrils protrude as lateral loops in all directions. This is how **chromonemes** (visible even with a light microscope), with diameters of 0.2 µm, are formed (see Sect. 2.2.3.7). The most extreme chromatin compaction is achieved by even more twisting of the metaphase chromosomes during mitosis and more so during meiosis (*Figs. 2.23*, *2.24*, *2.29d*, *2.35f–h*).

In contrast to the inactive condensed chromatin, **active chromatin** is maximally loosened. Histones are modified in the affected regions by methylation, acetylation, or phosphorylation, which reduces their affinity for DNA. The DNA itself is thus made somewhat more accessible for transcription factors and replication and transcription enzymes, and also particularly sensitive to DNase I. The **transcription factors** bind sequence-specifically to DNA regions and induce transcription (see Sect. 6.2.2.2).

2.2.3.2 Chromosomes and Karyotype

The term "chromosome" (derived from the Greek *chróma*, color, because of the easy stainability of condensed chromosomes) was first introduced over 100 years ago by the anatomist W. Waldeyer. Since DNA is recognized as the bearer of genetic information, this term has been applied to all gene-bearing structures, meaning that it is used with regard to mitochondria, plastids, bacteria, and even viruses, although histones are not involved in any of them, and the characteristic condensation/decondensation cycles are absent. The entirety of the genes or the gene-bearing structures of an organism is called the **genome** (Greek *génos*, origin, genus). In plants, as well as the **nucleome**, there are also the **plastome** (plastid genome) and the mitochondrial **chondrome** (chondriome; see Sect. 6.2.1), which are smaller than the nucleome (Fig. 6.4). These days, genome sizes are usually given by the total number of DNA base pairs (Table 6.2).

The chromosome complement in a cell nucleus in the members of a species is called their **karyotype**. It records all the cytologically identifiable chromosome features (size, form, number). The karyotype is an important genetic, systematic, and phylogenetic characteristic. The number of identical chromosome sets in a cell nucleus determines the level of **ploidy**, *n*. Cell nuclei with only one set of chromosomes are haploid (1*n*; Greek *haplós*, simple). Somatic (tissue) cells are predominantly diploid (2*n*) in ferns and seed plants. Nuclei of extraordinary size are mostly polyploid; they contain several to many copies of the gene and chromosome complement of the species in question. Even artificially generated polyploid cell nuclei result in enlarged cells. The ***C* value** refers to the total amount of DNA in the haploid genome, given in picograms. The *C* value of the bacterium *Escherichia coli* is 0.004, that of tobacco is 1.6, that of maize is 7.5, and that of some lily species can be over 30.

The individual chromosomes in a chromosome complement store different parts of the genetic information and, accordingly, often assume different forms (*Fig. 2.24*). The schematic representation of the simple haploid chromosome complement of a species is called a **karyogram** or **idiogram** (Box 6.1, Fig. B6.3, Fig. 9.9). Karyotyping is based on light microscope studies of that stage of cell division where the chromosomes are most condensed (metaphase, see Sect. 2.2.3.5). The following chromosome features are particularly important (*Fig. 2.24*): length, position of the centromere, presence or absence of a nucleolar organizing region (NOR), and heterochromatic sections. The **centromere** (primary constriction; Greek *kéntron*, middle point, and *méros*, part) is the narrowest point on the chromosome, where the chromosome bends during chromosome movements in cell division and where the microtubules of the nuclear spindle attach. These microtubules

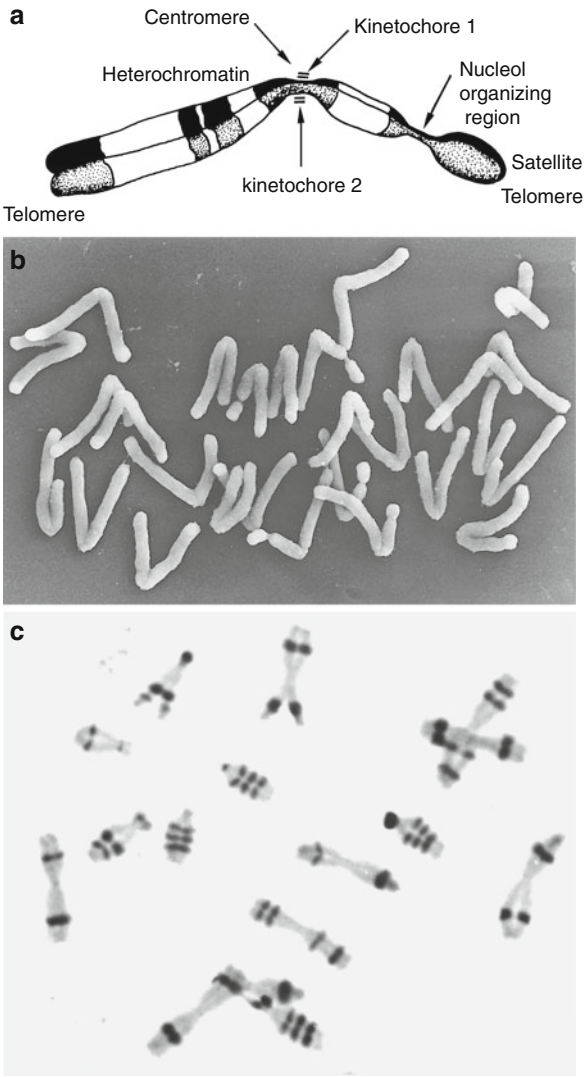

Fig. 2.24
Chromosomes occur as compact entities during nuclear division (e.g., in metaphase and anaphase of mitosis). This entity is what was originally named "chromosome." (a) A satellite chromosome with the two telomeres, the centromere with the two kinetochores (insertion points of the microtubules on the spindle apparatus), heterochromatic bands (additional regions on the telomeres and in the region of the centromere), and the characteristic (for satellite chromosomes) nucleolar organizing region (NOR) and a heterochromatic satellite. The chromosome is split longitudinally into two chromatids that later become daughter chromosomes. (b) Anaphase chromosomes of barley (*Hordeum vulgare*) with a diploid chromosome number 2*n* = 28, two satellite chromosomes per complement. The four NORs and four satellites of the two complements of daughter chromosomes can be seen clearly (×1,880). (c) Chromosome complement of *Anemone blanda* (2*n* = 16); heterochromatic bands (except at the centromere) picked out with color (×600) (b Specimen from R. Martin, SEM image from G. Wanner; c LM image from D. Schweizer)

end in a platelike or hemispherical multilayered structure that is located on the side of the centromere and is called the **kinetochore** (Greek *kínesis*, movement, and *chóros*, place). The centromere divides the chromosome into two arms, whose relative length can range from similar to quite disparate. The **centromere index** quantifies the length ratio of the arms (short arm length divided by the total chromosome length).

The chromosome ends are formed into **telomeres**. These inhibit the fusing of chromosomes that can occur after chromosomes have broken (see Sect. 6.2.1.1). Specific proteins can cause the telomeres to attach to the nuclear envelope. The telomere DNA is characterized by having huge numbers of sequence repeats. Replication of this DNA is performed by a specialized RNA-carrying **telomerase**. Microdigestion tests have shown that each chromosome has a single continuous strand of DNA (single-strand model); after replication in the S phase of the cell cycle, they have two strands. Since the advent of DNA sequencing, whole sequences of chromosomes and genomes have become available. In this way, many details of the chromatin's **sequence organization** can be determined, e.g., the relative position and particular structure of start sites for DNA replication (origins), coded and noncoded sequence sections, exons and introns, and regulatory and multiple sequences. These are treated further in Sects. 6.2.1 and 6.2.2.

2.2.3.3 Nucleoli and Preribosomes

The nucleoli are the sites of ribosome biogenesis. As compact, dense structures, they are easily recognizable under the light microscope (because of the high protein density). Each nucleolus contains a section of chromosomal DNA called the NOR (Nucleolus Organizer Region), which is composed of repetitive genes coding for all rRNAs but the 5S rRNA. Chromosomes with an NOR are called satellites or satellite chromosomes. During metaphase, the NOR can be seen as a constriction in a chromosome arm with the light microscope (*Fig. 2.24a, b*). It is called

a secondary constriction (the primary constriction is the centromere). There is always at least one satellite chromosome in haploid chromosome complements, and in plants, usually only one, meaning that the number of nucleoli represents the ploidy level: nuclei of diploid tissue cells contain two nucleoli, the triploid nuclei of angiosperm endosperm contain three.

The rDNA is an example of multiple sequences: numerous transcription units are laid down in tandem behind one another and are separated from each other by spacers – short noncoding regions. Each transcription unit contains the genes for the "large" rRNAs in the same order and is transcribed as a complete unit. The primary transcript – the pre-rRNA – is subsequently stripped down into the individual rRNAs and freed from the flanking sequences, riboresidues, and bases are methylated in places. The nucleolus thus has its own processing machinery that deviates from that of the nuclear space.

Ribosomal DNA (rDNA) is free from nucleosomes. Transcription is performed by the nucleolar **RNA polymerase I**, which is hardly sensitive to amanitine. The molecules of RNA polymerase I are thickly lined up on the transcription units; each unit is synchronously transcribed about 100 times. There is also huge repetition of the rRNA gene. High numbers of repeats are common in higher plants (wheat has up to 15,000 copies per nucleus, pumpkin up to 20,000, maize up to 23,000). This shows that the demand for ribosomes, especially in growing cells, is enormous. As ribosomes only exist for a few hours, the ribosome complement must be renewed continually. The size of the nucleoli reflects the intensity of protein biosynthesis in a cell. In cells that do not synthesize proteins (e.g., generative cells such as pollen tubes), the nuclei contain only small or even no nucleoli.

As the maturity of the transcripts increases, more ribosomal proteins arrive until the completed **preribosomes** detach from the nucleolus (as immediate predecessors of the large and small ribosome subunits) and are transported through the pore complexes into the cytoplasm.

The time these processes take is reflected in the **structure of the nucleoli** (Fig. 2.25). There are three different zones: (1) the rDNA of the NOR meanders through the nucleolus surrounded by loose fibrillar material (**pars fibrosa**, **fibrillary centers**) – these centers are the transcription sites; (2) the filamentous material thickens toward the outside into dense fibrillar zones, the processing centers; (3) finally, the periphery of the nucleolus is formed by a **granular zone**, the **pars granulosa**, in which the preribosomes accumulate.

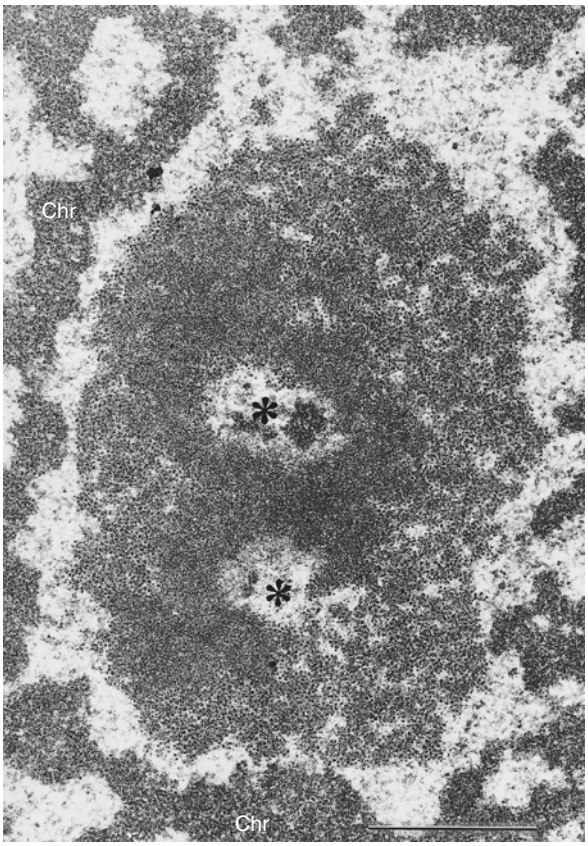

Fig. 2.25
Nucleolus in the nucleus of a cell from the root meristem of *Allium cepa* (onion). The NOR of the satellite chromosomes (*asterisks*) is surrounded by densely packed fibrillar material. It contains the primary transcripts, whereas the preribosomes are clustered in the outer granular zone. *Scale bar* 1 μm. *Chr* chromatin (EM image from H. Falk)

2.2.3.4 Nuclear Matrix and Nuclear Membrane

If the nuclear envelopes of isolated nuclei are destroyed by detergents and the soluble proteins carefully removed, even after nuclease cleavage, there remains a gel-like, loose structure that has the shape and size of the original nucleus. This **nuclear matrix** is made up of a mix of various proteins.

Chromosome regions involved in replication and transcription are tightly bound with it. The same is also true for DNA replication enzymes and for RNA polymerases. They are stuck to the nuclear matrix and pull the DNA along it. Immunomicroscopy has shown that transcription and processing are concentrated in particular positions in the nuclear space. Even the DNA itself has attachment sequences for the nuclear matrix at certain distances and forms loops between these fixed points. The loops, despite the linearity of the chromosomal DNA, behave as circular DNA. Transcription and replication in neighboring loops on the same chromosome can be regulated independently of each other.

Immediately inside the nuclear envelope the nuclear matrix thickens to form the **nuclear lamina**. This is only found in animal cells because plant cells lack all the intermediary filaments. The nuclear lamina is constructed from characteristic proteins, the **lamins**. The collapse of the nuclear envelope during nuclear division is initiated by strong phosphorylation of the lamins. The novel formation of the nuclear envelope during the formation of daughter nuclei is, in contrast, connected with lamin dephosphorylation. The remaining nuclear matrix also partly breaks down during nuclear division, leaving a significantly simpler chromosome skeleton in the chromosomes.

The **nuclear envelope** is linked to ER cisternae in several places and has ribosomes on its outside. This indicates that it is actually part of the ER that is indeed distinguished by its special location between karyoplasm and cytoplasm and by having **nuclear pore complexes** (**NPCs**; *Fig. 2.26*). The outward transport of RNAs (e.g., mRNA, tRNA) and ribonucleoproteins (e.g., ribosome subunits), the inward transport of karyophilic proteins (e.g., histones, DNA and RNA polymerases), and the repeated inward–outward migration of certain proteins and complexes that commute to and fro between the nuclear space and the cytoplasm space (e.g., importin, exportin; Fig. 6.17) take place through the nuclear pores. Nuclear import is regulated by the monomeric GTP-binding protein Ran (see Sect. 6.3.1.4). Up to 80 NPCs can be found per square micrometer of nuclear envelope. The complexity of the NPCs, which look very similar across all eukaryotes, is enormous (*Fig. 2.26b*). With a mass of over 100 MDa, they exceed the mass of a ribosome by a factor of 10–30. There are 30 core proteins (**nucleoporins**), and more than 100 additional proteins involved in their construction. Many nucleoporins contain the twin sequence of phenylalanine–glycine in multiple repeats. This implies a phyletic relationship between the nucleoporins.

2.2.3.5 Mitosis and the Cell Cycle

Mitosis (Greek *mítos*, thread) is the most frequent form of nuclear division (**karyokinesis**). The process produces two identical daughter nuclei from one cell nucleus. The name refers to the associated occurrence of condensed chromosomes. The first in-depth studies of mitosis were conducted by E. Strasburger, the founder of this book, and the anatomist W. Flemming. They studied plants and animals with especially long chromosomes (*Fig. 2.27*). Prior to each mitosis during **interphase** (the phase between two successive mitoses), the genetic information stored in every cell nucleus is replicated. Mitosis, with the aid of the **nuclear (mitotic) spindle**, is then the process by which the two sets of identical chromosomes are precisely uniformly distributed to the novel daughter nuclei. Genetically, mitosis is an **equational division** (Latin *aequális*, equal). All cells that have arisen from a single cell by mitosis are a cell clone; a mass of genetically identical cells (Greek *klon*, branch, shoot). Mutation can overrule the genetic unity of a clone. Mitosis is frequently but not always linked with a **cell division** (**cytokinesis**). Despite the equational division of the cell nucleus, this may be unequal and, e.g., result in two unequally large daughter cells. **Unequal (asymmetric) cell divisions** always take place at the start of differentiation processes.

There are no mitoses in prokaryotes. However, even there, although by quite different mechanisms, the equal division of the replicated genetic material into the daughter cells is guaranteed, also forming cell clones (see Sect. 2.3.1). DNA cloning, i.e., the identical multiplication of any DNA sequences in rapid-growing bacterial cultures, is a core method used in molecular biology.

The mitotic sequence has been known for about 100 years. It is usually described in five steps (*Figs. 2.27*, *2.28*). In a relatively long preparatory phase, **prophase**, during which the chromosomes slowly condense, the sensitive genetic material is transformed from its loose "working form" to its compact "transport form" (*Fig. 2.29*). This is visible using light microscopy as an enlargement of the chromatin structure, and the chromosomes become individually visible. In places, their arms seem to be split lengthways. The DNA replication that has already occurred also becomes manifest in the chromosome structure. The chromatin condensation is

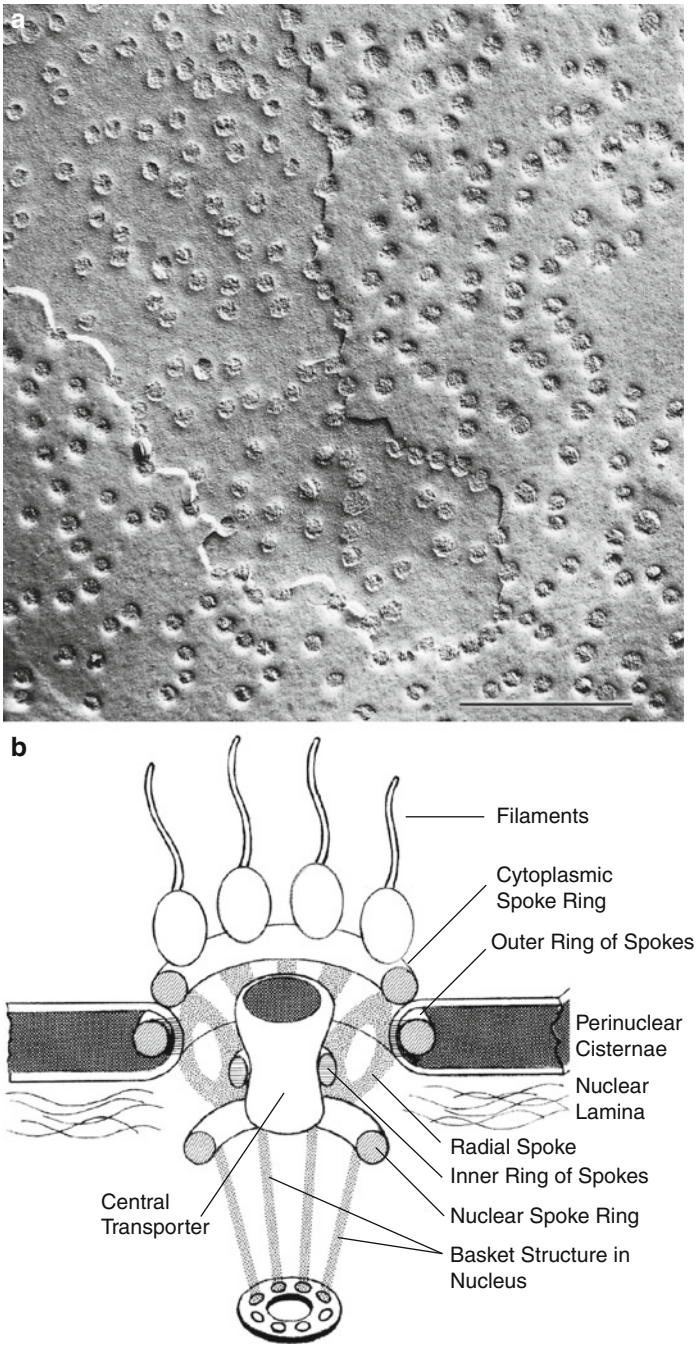

Fig. 2.26

Pore complexes in the nuclear envelope. (a) Nuclear envelope of *Allium cepa* (onion), freeze fracture (*scale bar* 1 μm). (b) Ultrastructure model of a pore complex. In the perinuclear cisternae, there is an outer ring of spokes that, together with the nuclear and cytoplasmic spoke ring, bear the radial spokes. The regions between the spokes are sealed/insulated with amorphous material. The cytoplasmic ring of spokes bears eight particles, from which filaments stretch into the cytoplasm. The spokes support a central tube-shaped plug (central granula) over the inner spoke ring. Various particles are exchanged between the nucleus and the cytoplasm (a EM image from V. Speth)

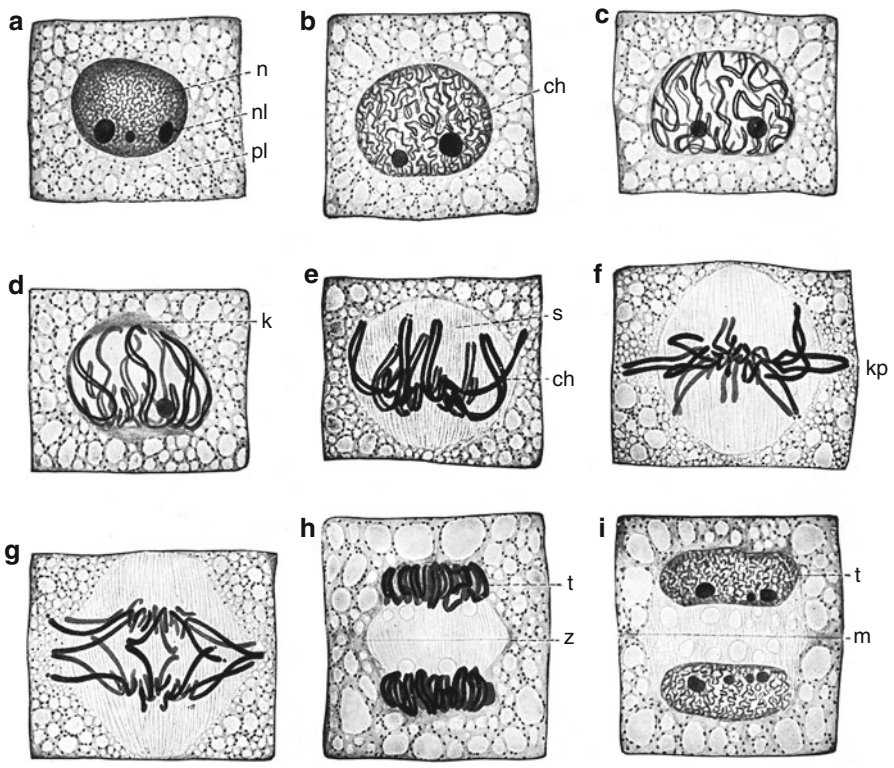

Fig. 2.27
Mitosis and division of an embryonic cell (root tip of *Aloe thraskii*) (×1,000). (a) Interphase. (b–d) Prophase. (e) Prometaphase. (f) Metaphase. (g) Anaphase. (h, i) Telophase, and cell division. *n* nucleus, *nl* nucleolus, *ch* chromosomes, *pl* cytoplasm, *s* spindle, *k* pole caps, *kp* equatorial plate, *t* daughter nuclei, *z* growing cell plate in phragmoplasts, *m* cell plate, which later becomes the middle lamella of the new cell wall (After G. Schaffstein)

caused by proteins among which the linker histones of the H1 group and the so-called SMC proteins play a major role. (The acronym SMC is based on the *SMC1* gene discovered in *Saccharomyces* yeast whose product causes the *s*tabilization of *m*ini *c*hromosomes.)

The spindle apparatus forms in the cytoplasm during prophase. Even before the chromatin has condensed, the peripheral microtubules have, in many cases, combined to form a **preprophase band**, which marks the future position of the cell equator in the plant cell (*Fig. 2.13*). Later, the microtubules arrange themselves into characteristic **mitotic spindles** (*Box 2.2*). The larger cytoplasm organelles are pushed out of the spindle area. End of prophase is marked by the fragmentation of the nuclear envelope. The perinuclear cisternae disintegrate into vesicles and small cisternae that are moved to the spindle poles. They are later pulled in to form the new envelopes of the daughter nuclei.

Prophase is followed by a transition phase in which the microtubules of the spindle apparatus contact with the chromosome kinetochores and migrate to the cell equator – the symmetrical plane between the spindle poles (**metakinesis** during **prometaphase**). Immediately following the fragmentation of the nuclear envelope, the nucleoli are freed from the secondary constriction by the satellite chromosomes and migrate away from the spindle area. They tend to dissolve in the cytoplasm. Part of the nucleoli material becomes adsorbed to the chromosome surfaces and is later transported into the daughter nuclei.

The centromeres are characterized by DNA sequence repeats (often quite numerous) that are never transcribed. This is also the location of many specific proteins (centromere proteins) that among other things build the platelike kinetochores and anchor them to the chromomere DNA. The outer kinetochore plate has a high affinity for the plus ends of the spindle microtubules, and the inner plate has high affinity for the centromere chromatin.

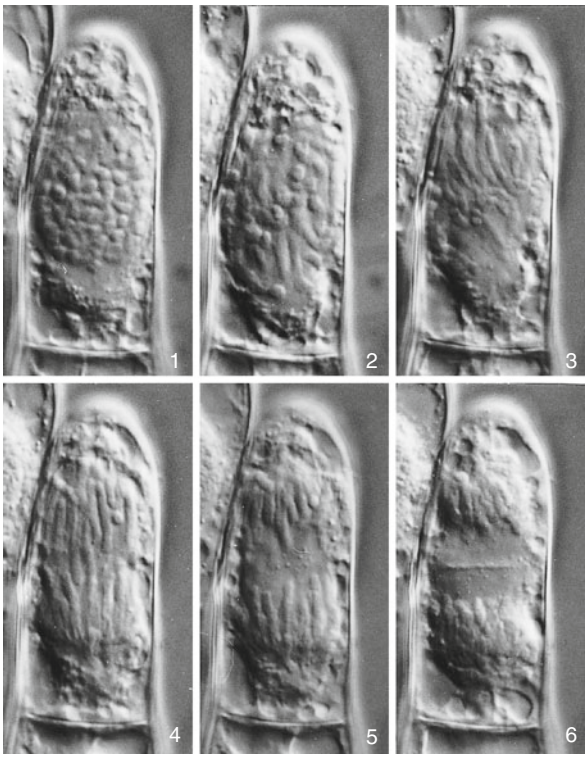

Fig. 2.28
Mitosis and cell division in the end cell of an anther filament hair of *Tradescantia virginiana*, live specimen (×680). *1* end of prophase, terminal caps clear above and below the condensed chromosomes, *2* prometaphase (metakinesis, duration 15 min), *3* metaphase (15 min), *4*, *5* anaphase (10 min), *6* beginning of telophase, and cell division by cell plate formation (Differential interference contrast images from Hepler 1985, with permission from Rockefeller University Press)

Bit by bit, the centromeres of the by now maximally condensed chromosomes arrive at the cell equator, and the arms hang toward the poles from the equatorial or metaphase plate. This is then **metaphase** (Greek *metá*, between). This is the phase when the entire chromosome complement can be observed using light microscopes (*Fig. 2.24b, c*). With use of the alkaloid colchicine, which causes the breakdown of the labile spindle microtubules, the mitosis can be arrested in metaphase.

Metaphase lasts a relatively long time. This allows the correct alignment of the only slightly swaying chromosomes in the spindle apparatus. At the same time, the final division of the replicated chromosomes is prepared for, and the future daughter chromosomes become clearer as lengthways halves of the chromosomes (chromatids). Eventually, the **chromatids** are often only attached at the centromere. They are held together by **cohesin**, a multimeric protein complex. **Anaphase** (Greek *aná*, up) begins abruptly with the synchronous proteolytic breakdown (via the activation of the anaphase-promoting complex, a ubiquitin E3 ligase) of the cohesin complex. The now independent daughter chromosomes begin to migrate toward the spindle poles with the help of the mitotic spindle (*Box 2.2*). In the process, one daughter chromosome is pulled to one pole, and the other daughter chromosome is pulled to the other pole: anaphase is where the genetic material is distributed to the future daughter nuclei and cells. The still undivided cell has a higher ploidy level during this phase. If the nucleus had been diploid ($2n$), then the cell would now be temporarily tetraploid ($4n$).

This can be useful in the cultivation of polyploid plants. Treatment of the vegetative points with colchicines causes many tetraploid cells to be formed in the shoot meristems. The separated chromosomes are, under the continued disintegration of the spindle apparatus, forced into a single restitution nucleus that is accordingly much larger and which remains tetraploid during subsequent mitoses. The cell size, and in plants of economic value also the harvest, increases owing to the altered nucleus-to-plasma ratio.

A consequence of the anaphase migrations is that the two daughter chromosome sets in the still undivided mother cell are pushed apart as far as possible. The chromosomes shift finally stops, and the end of anaphase, the shortest phase in mitosis, has been reached.

The closing phase (**telophase**; Greek *télos*, end) sees the significant prophase processes run in reverse order and direction. The spindle apparatus decays, and nuclear envelopes form around the chromosomes squeezed into the two pole regions by the fusion of ER cisternae. Nuclear pores soon develop. The chromosomes loosen up and the euchromatic parts change into the typical interphase chromatin, becoming physiologically active once again. The nucleoli are also reformed rapidly, initially by condensation of material that was on the chromosome surfaces but also by resumption of the synthesis of RNA precursor stages in the NOR of the satellite chromosomes. Protein synthesis in the cytoplasm that had been arrested during mitosis begins again, and cell division is completed (see Sect. 2.2.3.6). When telophase finishes, **interphase** begins – the actual working phase of chromatin. It lasts significantly longer than a whole mitotic event.

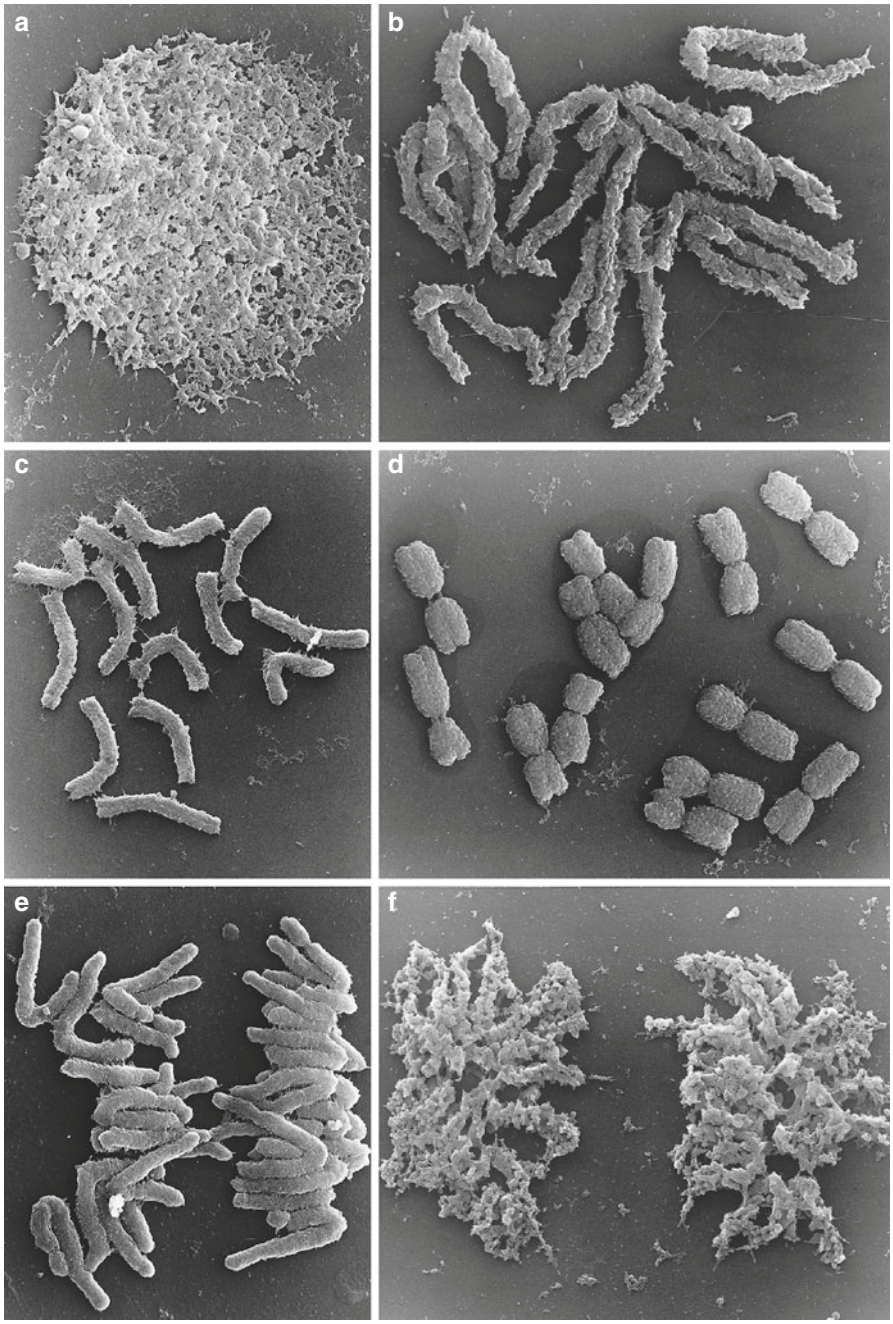

Fig. 2.29

Chromatin condensation and decondensation during mitosis. (a) Interphase. (b, c) Increasing condensation during prophase and metakinesis. (d) Metaphase (arrested by amiprophos methyl, which causes particularly strong condensation). (e) Anaphase. (f) Decondensation during telophase. The specimen was extracted from cell suspensions prepared from fixed root tips of barley (*Hordeum vulgare*) by enzymatically digesting the cell walls. The protoplasts burst when dropped onto a cold slide. After cover slips have been covered, the specimens are frozen, the cover slips are then removed, and the material is dehydrated and examined under an SEM (After G. Wanner)

Box 2.2: The Nuclear Spindle

The chromosome movements during mitosis and meiosis are mainly driven by the nuclear spindle. It is built de novo for every nuclear division and then broken down again.

The **spindle fibers** seen in good conditions using the light microscope are actually (using the electron microscope) bundles of microtubules of the labile type. *Figure B2.2* shows a diagrammatic representation of the three different (according to position and function) types of microtubule components in the nuclear spindle. The entire spindle apparatus is a bipolar structure with mirror-image symmetry made up of two antiparallel half-spindles. The two spindle poles function as MTOCs (see Sect. 2.2.2.1). They produce:

- Kinetochore microtubules which extend to the centromeres of the chromosomes and assume contact with three-layered anchorage plates, the kinetochores. Spindle fibers used to be called chromosome fibers or traction fibers.
- Pole microtubules (used to be called continuous fibers or pole fibers), which are pulled toward the spindle equator and form an overlap zone along the symmetrical plane of the spindle. The phragmoplast arises in this region during telophase (see Sect. 2.2.3.6).
- Aster microtubules, which do not migrate to the kinetochores or the overlap zone but radiate out in various directions from the poles. The asters (Greek *ástron*, stars) can be quite developed in some animal cells. They surround the centriole pairs located at the poles like a densely rayed corona. This spindle component is often rather poorly developed in plants and is sometimes lacking altogether.

The spindle apparatus has ER spun around it, and appendages of this membrane system extend in-between the spindle microtubules (mitotic reticulum).

During prophase, the spindle microtubules form around the cell nucleus. Under the light microscope, flat double-refracting surfaces become visible immediately outside the nuclear envelope, from which all large cell organelles are excluded (terminal caps or **attraction plates**). In contrast to most animal cells, the centrioles of plant cells hardly play a role at all. This is true not only for angiosperms, which have no centrioles, but also for many gymnosperms and even for some fungi and algae (at least in the vegetative stages). Dense plasma zones without distinct boundaries are found at the spindle poles and are termed **centroplasm** (they are often called **centrosomes**. Beware! Do not confuse them with the centromeres of the chromosomes). They function as MTOCs.

In anaphase, two movements occur more or less synchronously. The centromeres of the daughter chromosomes migrate toward the poles as the kinetochores shorten (**anaphase A**) and the poles move apart (**anaphase B**). Both migratory processes occur slowly but constantly, with speeds on the order of 1 μm min^{-1}. Together they ensure the greatest possible distance is put between the two sets of daughter chromosomes.

Because only the spindle poles function as MTOCs, in the spindle apparatus all microtubules are aligned so that their minus ends lie at the poles and the plus ends lie at the equatorial overlap zone and at the kinetochores. The microtubules of both half-spindles are arranged antiparallel to each other in the overlap zone. Here, during anaphase, a kinesin-like ATPase causes the opposing-oriented microtubules to slide apart and thus a pushing apart of the two half-spindles (the effect of pole microtubules as "motor bodies"; anaphase B). The mechanism of anaphase A is less well understood. Inhibitor studies have shown that the actomyosin system of the cell is not involved. Surprisingly, the kinetochore microtubules are not transported away during anaphase and do not shorten at the poles, but at the kinetochores, at their plus end. Cytoplasmic dynein is also concentrated here. The role of local peaks in Ca^{2+} concentrations (the ions are released from the mitotic reticulum) during breakdown of the kinetochore microtubules remains unclear. Calmodulin is bound to kinetochore microtubules, whereas polar microtubules are free of it.

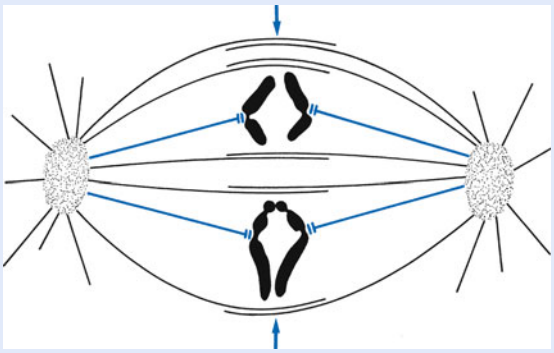

Fig. B2.2
Nuclear division spindle in early anaphase. *Black* aster microtubules and polar microtubules, *blue* kinetochores and kinetochore microtubules, *arrows* equator

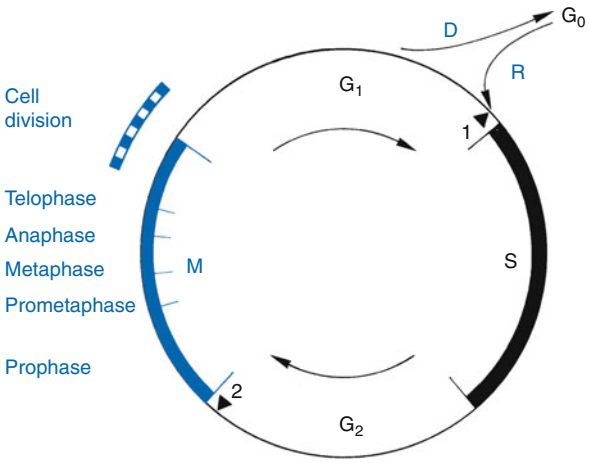

Fig. 2.30

Phase succession in the cell cycle. *M* mitosis, G_1 postmitotic growth phase, *D* differentiation into tissue cells, whose DNA remains unreplicated (G_0), *R* Reembryonalization, e.g., during regeneration, *S* replication of DNA, G_2 premitotic phase, *arrowheads 1* and *2* control points. The complex regulation of the cell cycle is shown in Fig. 6.19

The regular succession of mitosis and interphase is called the cell cycle (*Figs. 2.30*, 6.20). Meristematic cells constantly experience the cell cycle. As they become tissue cells, after a final mitosis, the cycle stops. Isotope studies have shown that replication of chromosomal DNA occurs midway through interphase. This is called **S phase** ("S" for synthesis of novel DNA). The phase between mitosis (**M phase**) and S phase is called **G_1 phase** and that between S phase and the next mitosis is **G_2 phase** ("G" for gap). Successive cell cycles continuously alternate replication and segregation of the genetic material. The intermediate G phases allow the growth of the cell (especially in G_1 phase) or the preparation for the next mitosis (G_2 phase). A decisive checkpoint is found before the start of S phase. If it is exceeded, then the cell affected must undergo another mitosis, i.e., to complete another cell cycle. If the checkpoint is not exceeded, then the cell and nucleus divide no further, and they differentiate into a tissue or permanent cell (**G_0 phase**).

Sometimes, there are strong deviations from the normal course of the cell cycle. Although G_1 phase is generally the phase for embryonic cell growth (not by vacuole enlargement, but based on protein and membrane synthesis), it can be significantly shortened or even absent in cases of very rapid cell replication. This is,

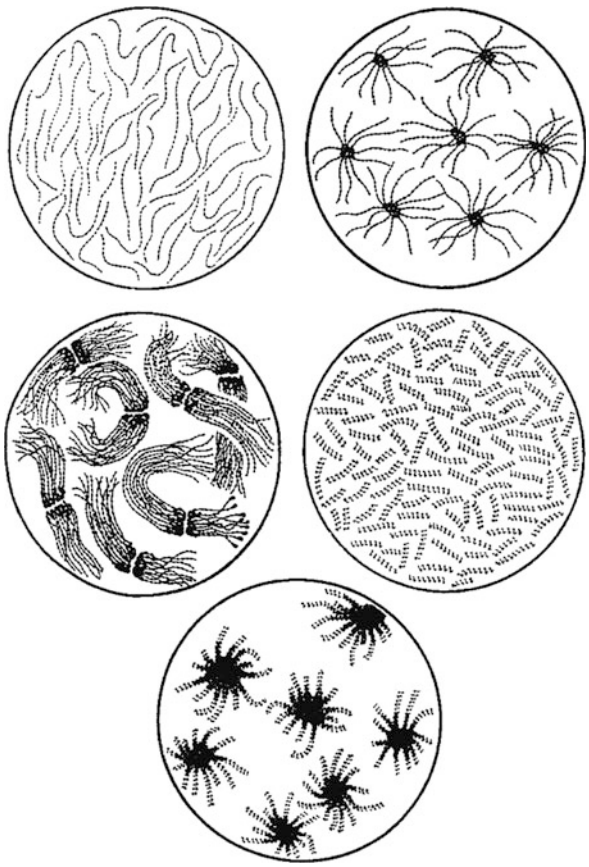

Fig. 2.31

Different chromatin structures of endopolyploid nuclei in the antipodal cells (embryo sac) of the common poppy (*Papaver rhoeas*) (After G. Hasitschka)

e.g., the case in the slime mold *Physarum*. All nuclei in its multinucleate plasma mass undergo synchronous nuclear division; plasma replication occurs in G_2 phase. An even greater deviation from the normal cell cycle results in endopolyploid cells: repeated S phases occur without switching over to M phases. (The usual term "endomitosis" is rather misleading.) The regulatory processes of the cell cycle (**cell cycle controls**) are dealt with in Part II of this book (see Sect. 6.3.2).

The endopolyploid saliva glands of many insects (especially dipterans) have the well-known polytene **giant chromosomes** with their characteristic banding. Similarly spectacular formations are rather unusual in plants even though endopolyploid cells are not uncommon, and occur regularly in regions such as the embryo sac (*Fig. 2.31*).

2.2.3.6 Cell Division

Cell divisions are usually associated with nuclear divisions. During telophase, while the spindle apparatus is broken down, new, relatively short microtubules are formed in great numbers at the equator of the cell that are oriented perpendicularly to the equatorial plane. The regular alignment of the microtubules results in the plasma zone between the daughter nuclei becoming double refractive. This is called the **phragmoplast** ("wall former" from the Greek *phrágma*, boundary, and *plástes*, former). Actin filaments accrete here and many active dictyosomes concentrate around the phragmoplast. Golgi vesicles filled with cell wall matrix migrate from the dictyosomes into the phragmoplasts, arrange themselves in the equatorial plane, and fuse together after forming special fusion tubules. This forms the first wall layer – the **cell plate** – between the daughter cells. The formation process generally begins in the middle of the former mother cell and the cell plate grows toward the wall of the mother cell as further Golgi vesicles are incorporated at the edges. This process is usually quite quick, and the separation of the daughter cells only takes a matter of minutes. In large cells, e.g., in the cambium initials (*Fig. 2.32*; see Sect. 3.1.2), this process of centrifugal growth can take significantly longer. Even during its formation, the cell plate becomes traversed by ER tubes around which the first plasmodesmata are formed. As soon as the mutual delimitation of the daughter cells is completed, in each daughter cell, the parting of the first lamellae from its own primary cell wall starts; the primary cell wall already contains a few cellulose fibrils.

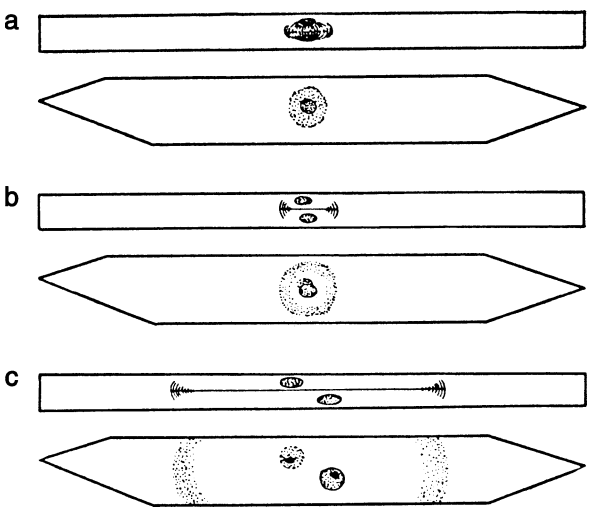

Fig. 2.32
Formation of the cell plate in a cambium cell. (a) Telophase, formation of the phragmoplasts. (b, c) The phragmoplast grows centrifugally and initially reaches the lateral walls of the elongated cell – the cell ends are still undivided (After I.W. Bailey)

Cytokinesis does not always follow on from karyokinesis. The result of these "free" nuclear divisions is multinucleate cells or **plasmodia**. These can reach macroscopic proportions (*Fig. 2.9*); palm-sized plasmodia of the slime mold *Physarum polycephalum* contain about one billion cell nuclei. Plasmodia are not uncommon in algae (e.g., siphonal green algae) and fungi (*Oomycota*, *Chytridiomycetes*, and *Zygomycetes*) and occasionally even occur in higher plants. For example, the "nuclear" endosperm of some seeds is a plasmodium (a well-known example is coconut milk), as are the multinucleate, nonsegmented lactifers of *Euphorbia*. Nuclear endosperm can change into cellular endosperm by free cell formation (*Fig. 2.33*). However, multinucleate cells (**coenoblasts**) can also arise through the fusion of uninucleate cells. These are then called **syncytia**. Examples of syncytial coenoblasts can be found among the segmented lactifers of dandelions (*Taraxacum*) or in the tapetum of pollen sacs.

Like in mitosis, cell division in the lower plants and fungi differs quite significantly from the textbook model. Flagellates and some algae show the same splitting process as seen in animal cells: binary fission (strangulation) of the mother cell by an equatorial actomyosin ring. In yeast, the mother cell is not divided at all; one of the two daughter cells is pushed into a preformed cell outgrowth that later buds off (cell budding; *Fig. 2.34*). In basidiomycetes, the hyphal cells containing nonmitotic nuclei of the dikaryotic stage divide by forming lateral outgrowths (Fig. 10.59); the two nuclei divide synchronously and in parallel, one in the hyphal cell itself and the other in the clamp. This ensures that each daughter cell contains a pair of genetically identical nuclei.

2.2.3.7 Meiosis

In mitosis both daughter nuclei get the same complement of genetic information, which is also identical to that of the mother cell. In contrast, **meiosis** in a diploid cell results in four haploid daughter nuclei acquired by a process of two successive divisions. These nuclei are not genetically identical to each other nor are they genetically identical to the mother cell. **Syngamy**, the fusion of

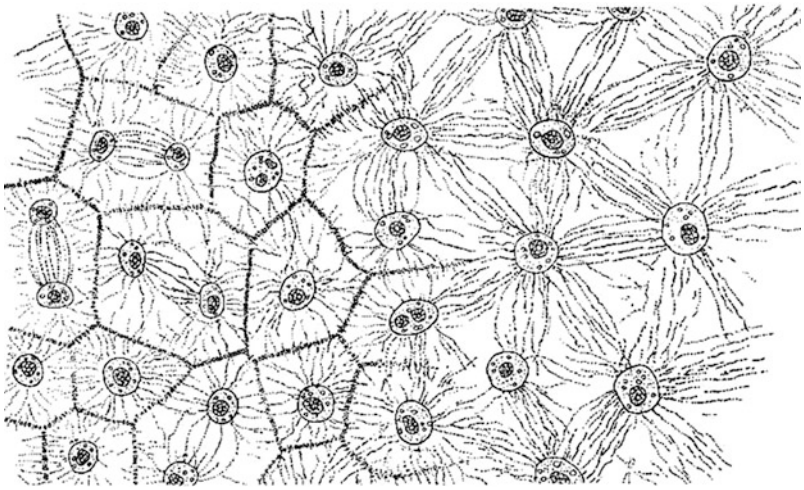

Fig. 2.33
Polynuclear endosperm of *Reseda* with (to the *right*) advanced cell wall formation (×240) (After E. Strasburger)

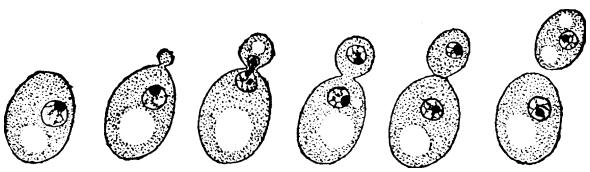

Fig. 2.34
Cell budding in brewer's yeast (*Saccharomyces cerevisiae*) (×100) (After A. Guilliermond)

two haploid, conspecific but genetically different **gametes** (Greek *gamétes*, husband/wife), results in a diploid cell with two similar but not identical chromosome sets – the zygote (Greek *zýgios*, united). Syngamy is the central cellular process of fertilization. Meiosis and syngamy are fundamental to **sexuality** in the scientific biological sense.

Sexuality enables the independent arbitrary combination of alleles (resulting from mutation) of different organisms of the same species. Selection then acts on advantageous, neutral, or deleterious combinations. This is what the selective advantage of sexual reproductive cycles in evolution is based upon, and which is particularly emphasized in large genomes, i.e., those of complex multicellular organisms.

The precision of DNA replication and the chromosome assortment by the spindle apparatus rule out chance events that may disrupt mitosis.

Sexual processes, on the other hand, give chance an opportunity. In an entire sexual reproductive cycle there are three points at which chance generators are built in:

1. During meiotic prophase (see below), there is **intrachromosomal recombination** – multiple section exchanges between paternal and maternal chromosomes of the diploid complement; the location and extent of these reciprocal exchange events are mostly random.
2. During the first meiotic division, maternal and paternal chromosomes are randomly assigned throughout the daughter cells (**interchromosomal recombination**).
3. During the fusion of gametes which gametes actually fuse to form a zygote is also random.

Meiosis used to be called a **reduction division** because the diploid ($2n$) complement of chromosomes was reduced to a haploid ($1n$) one. However, the reduction from $2n$ to $1n$ could occur in a single step. In fact, after the first meiotic division (meiosis I) both daughter cells are already haploid. But, wherever in the world of organisms meiosis occurs, **meiosis I** is always followed by **meiosis II**, whereby four haploid cells arise. The following shows that it is only after the latter stage that the recombined genetic material becomes completely effective. Thus, meiosis is not, strictly speaking, only a reductional division, but is also, principally, a **recombination division**.

Meiosis and syngamy, the complementary fundamental genetic and cellular processes of every sexual reproduction, enable the continuous mixing of the **gene pool** (genes and alleles) of a species, which can be defined as a virtual reproductive community. (Alleles are different variants of a gene that take the same

position in homologous chromosomes and under whose influence a character may be differently expressed; Greek *alloios*, different).

Meiosis begins with a complex, temporally extended **prophase**. Prophase has many different stages because the chromosomes in the intact nuclear envelope become visible under the light microscope and undergo a series of characteristic changes (Fig. 2.35a–e).

During the **leptotene** (Greek *leptós*, thin, *tainía*, band, and *néma*, thread; after an extended premeiotic S phase and nuclear enlargement) the chromosomes become visible as delicate **chromonemes**. In many places, each

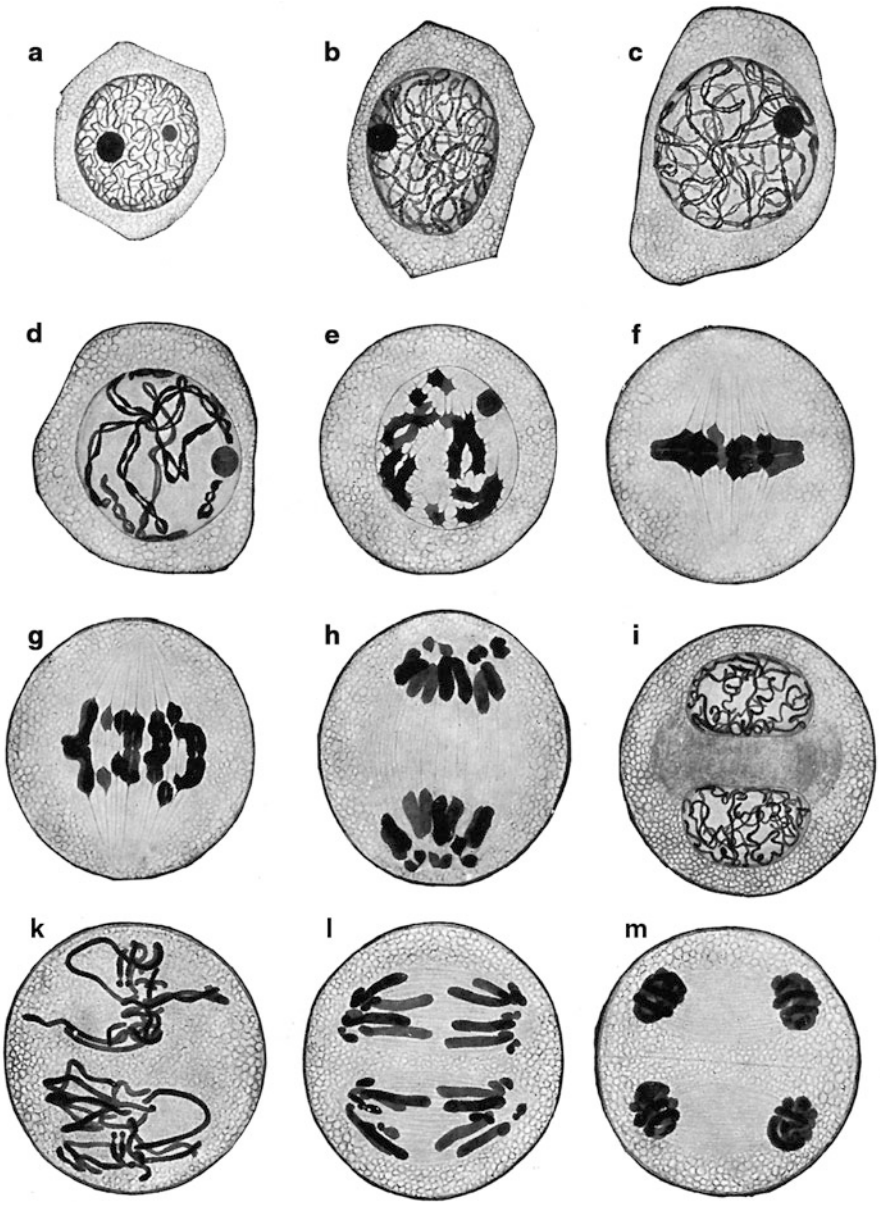

Fig. 2.35

Meiosis in pollen mother cells of *Aloe thraskii* (×1,000). (a–e) Prophase of meiosis I (*A* leptotene, *B* zygotene, *C* pachytene, *D* diplotene, *E* diakinesis). (f) Metaphase I. (g) Anaphase I. (h) Telophase I. (i) Interkinesis. (k–m) Meiosis II, formation of the four haploid nuclei (After G. Schaffstein)

characteristic for every chromosome, the chromonemes are balled up into **chromomeres** (Fig. 2.36). The telomeres of each chromosome are fixed to the nuclear envelope or nuclear lamina.

During the **zygotene** homologous chromosomes from the maternal and paternal complements pair up lengthways against each other (**synapsis, syndesis**). Normally, synapsis starts at the telomeres and continues zipper-like to the centromeres. Interlocking of another chromosome between the chromosome pair is extremely rare. This presumes the chromosomes have been appropriately arranged in the interphase nucleus, which is achieved by adhesion of the telomeres to the inner surface of the nuclear envelope and the subsequent pushing together of the chromosomes. **Synaptonemal complexes** (easily visible under the electron microscope) form between the paired homologs. These are protein structures that stabilize their cohesion (Fig. 2.37).

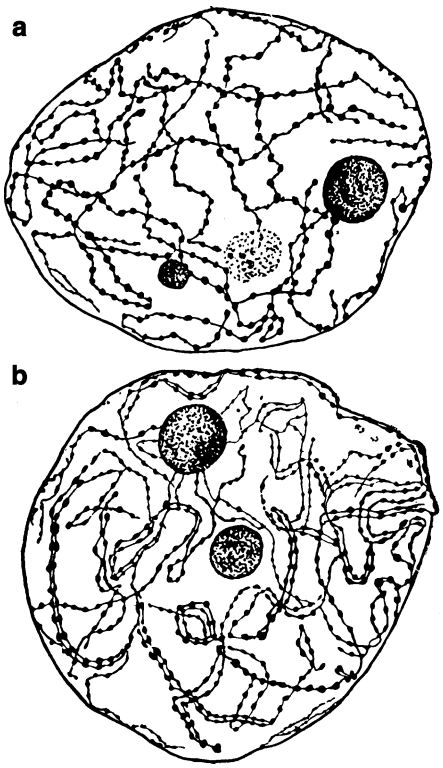

Fig. 2.36
Leptotene (a) and zygotene (b) in a pollen mother cell of *Trillium erectum* (×1,500). In homolog pairing, similar chromomeres lie alongside each other: "stepladder aspect" (After C.L. Huskins and S.G. Smith)

Pairing of homologs is completed in the **pachytene** (Greek *pachýs*, thick; Fig. 2.38). The number of chromosome pairs (bivalents) in the nuclear space is the same as the haploid chromosome number *n* of the species. Intrachromosomal recombination takes place in this phase. This is expressed as a temporary increase in reparative DNA synthesis and morphologically as **recombination knots** – dense, spherical structures of about 100-nm diameter that are located to the sides of the synaptonemal complex. The actual molecular exchange process, the **crossing-over**, remains unseen.

Bit by bit the chromosomes shorten by condensation and become thicker. This is in preparation for the next phase, the **diplotene**. Its start is marked by the end of synapsis, the synaptonemal complexes disappear, and the homologs begin to separate. They do, however, remain attached to each other at the points where crossing-over has occurred. The crossing-over is called a chiasma (after the Greek letter *chi*, χ). Each **chiasma** is a crude expression of the molecular crossing-over which forms the basis of intrachromosomal recombination (see Sect. 2.2.3.8). The chromosomes shorten even further and it becomes obvious that they have already replicated: each chromosome is split lengthways into two chromatids; the bivalents have become tetrads. Closer observation shows that of the four chromatids in a homologous pair only two of them are actually crossed over at a chiasma (Fig. 2.39d–f).

For cells the diplotene phase is often a growing phase and accordingly it can be a long-lasting phase. Cell growth generally requires increased transcription, and indeed diplotene chromosomes are often looser (**strepsitene**; Greek *streptós*, fringed, curly).

Diakinesis is the last stage of meiotic prophase. Transcription activity vanishes, and condensation of the chromosomes is maximal; they are even shorter and thicker than in mitotic metaphase. The undivided centromeres of each pair of homologs separate from each other as far as possible. This moving apart is restricted by the adjacent chiasmata. However, the chiasmata are often pushed in the direction of the telomeres, which are no longer attached to the nuclear envelope, and their number is reduced stepwise (terminalization of the chiasmata; Fig. 2.40).

Diakinesis (and thus meiotic prophase overall) is ended when the nuclear envelope fragments. During **metaphase I** the pairs of homologs arrange themselves at the spindle equator. The homologs are still attached to

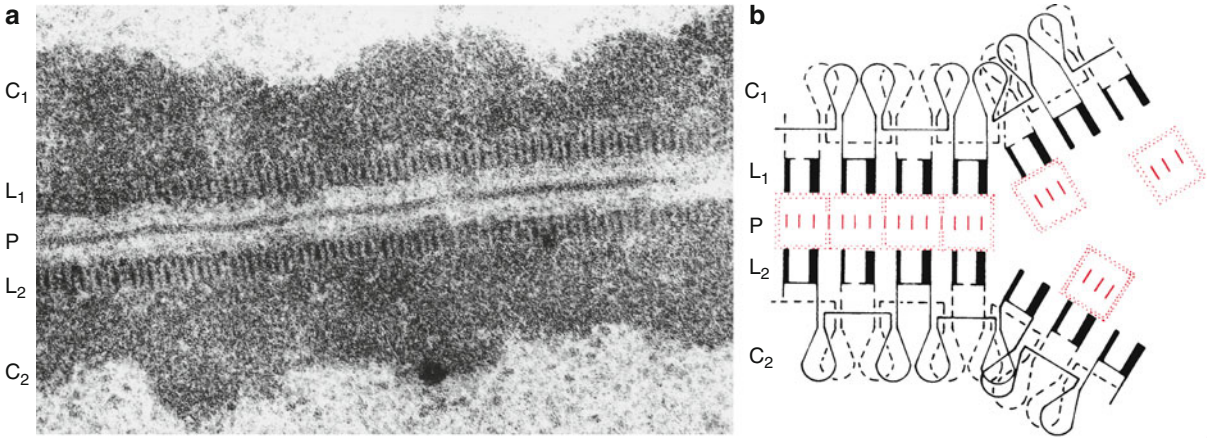

Fig. 2.37

Synaptonemal complex between paired chromosomes C_1 and C_2 in the ascomycete *Neottiella*. (a) Longitudinal section in an EM. (b) Diagram. Even before the start of pairing, the replicated chromosomes are unilaterally coated with transverse synaptomeres that form regularly spaced band-shaped lateral elements (L_1, L_2). The lateral elements of homologous chromosomes are bonded together by proteins with strong aggregation tendencies during zygotene; a dense central element (*P*) flanked by indistinct transverse elements is formed. In the synaptonemal complex, there are occasional molecular pairs of homologous DNA sequences each having two of the four chromatids. This is a prerequisite of intrachromosomal recombination via crossing-over (After D. von Wettstein)

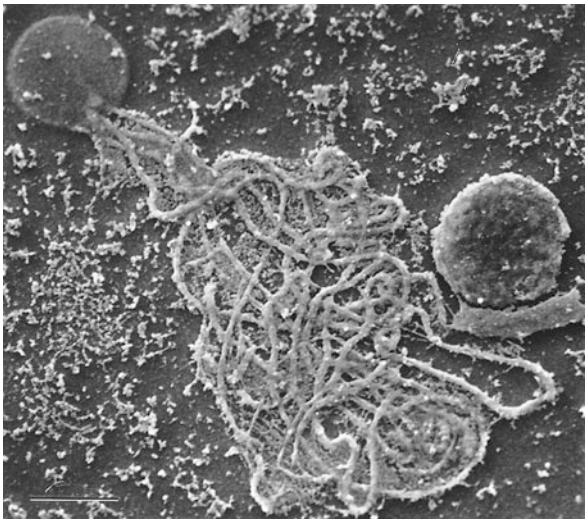

Fig. 2.38

Paired homologous chromosomes (bivalents) of rye *Secale cereale* (anthers, early pachytene). The bivalents have escaped from the burst prophase nucleus (*upper left*); to the *right*, there is an entire nucleus. *Scale bar* 20 μm (SEM image from G. Wanner)

each other by the chiasmata, or often only by the telomeres. A single kinetochore is found at the centromere of each chromosome. It is a matter of chance which of the two chromosomes of a pair of homologs goes to which spindle pole. This is fundamental to interchromosomal recombination.

The number of possible distribution patterns for maternal and paternal chromosomes in anaphase I, or the combination patterns of these chromosomes in the daughter cells, is 2^n. In an organism with $n = 10$ chromosomes in the haploid complement, there are over 100 possible combinations, with $n = 23$ (as in humans), there are 8.4 million possible combinations, and for $n = 50$, there are more than 10^{15}. The chance of a gamete having only paternal or maternal genes is extremely small just because of the random assignment of maternal and paternal chromosomes, and when segment exchange is factored in, the chance becomes nearly zero. The mixing of allele stock is extremely effective, even without taking syngamy into consideration.

During **anaphase I** the chiasmata are finally broken down, and the homologous chromosomes are no longer attached to each other and migrate apart in the spindle apparatus. Unlike in mitotic anaphase, the daughter cells

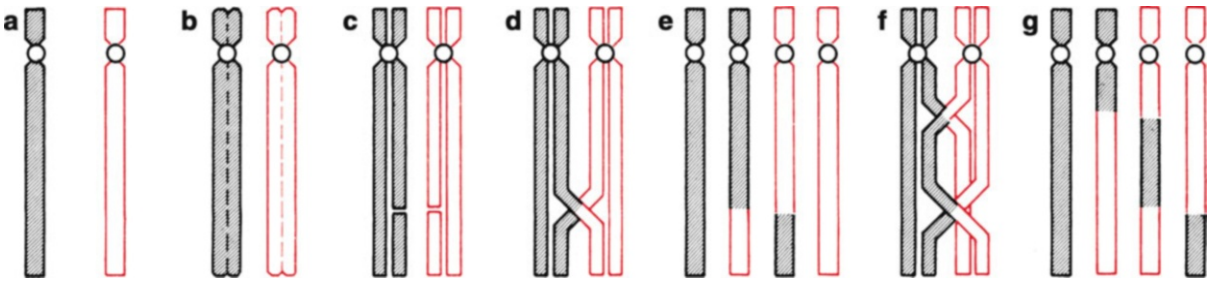

Fig. 2.39

Chiasma genesis. (a, b) Homolog pairing. (c) Genesis of corresponding chromatid bridges and (d) cruciform healing of two homologous chromatid sections. (e) Before reduction for the neighboring ("proximal") chromosome to the centromere sections; the "distal" sections (beyond the chiasma) after reduction. (f, g) Double crossing-over with triple-strand exchange, where the second exchange takes place between a chromatid already involved in the first exchange and a hitherto uninvolved one. Only two (always one maternal and one paternal) of four chromatids are ever involved in a crossing-over event (After R. Rieger and A. Michaelis)

Fig. 2.40

Decrease in the chiasmata number between the end of pachytene (a) and metaphase I (e). (*Anemone baicalensis*, ×1,000) (After A.A. Moffett)

do not acquire chromatids or daughter chromosomes but acquire already replicated chromosomes whose centromeres are not yet split and whose kinetochores have not yet replicated. These chromosomes are equivalent to the prophase chromosomes of a normal mitosis, not telophase chromosomes. The daughter cells, the so-called meiocyte I cells, have the haploid chromosome complement in their cells but have double the DNA quantity ($2C$) of the haploid, nonreplicated genome (C).

During meiosis II the C value is reduced from $2C$ to $1C$. There is no S phase or DNA replication in interphase between the first and the second meiotic division, the **interkinesis**. Interphase is therefore often quite short and sometimes is even omitted altogether. Only the kinetochores are replicated. During meiosis II, the chromatids that were formed during premeiotic S phase and underwent crossing-over during the pachytene are finally separated and each enclosed in separate haploid nuclei. Outwardly, meiosis II is like a haploid mitosis, but the sister chromatids of each chromosome are not identical in terms of their allele content. As a consequence of the interchromosomal recombination during the pachytene in meiotic prophase, the chromatids have repeatedly acquired a series of either paternal or maternal alleles at different gene locations. This is the case wherever an uneven number of crossing-over events have taken place between the centromere and the gene position in question. These frequently nonidentical sequences are separated from each other together with the identical ones from the original sister chromatid

(**postreduction**; *Fig. 2.39*). The haploid gametes contain only one allele of every gene. This guarantees that in subsequent mitoses, all the descendants of a chromosome are identical.

2.2.3.8 Crossing-Over

Certain DNA sequences of genes are held together within a chromosome or chromatid by intramolecular bonds along the DNA double helix. Therefore, in genetics, all genes located on a particular chromosome are indicated as being coupled or linked. A chromosome is the structural representation of what genetics calls a linkage group. The coherence of genes in a linkage group is disrupted when a crossing-over event occurs in the group. Nonsister chromatids of paired homologous chromosomes exchange sections. This process is induced during the pachytene, whereby endonucleases in the DNA double helices of two neighboring nonsister chromatids cause single- or double-stranded breaks and cross over, healing by ligation (*Fig. 2.41*). The process is often made more complicated because the single-strand breaks do not occur at exactly the same position, meaning that extra repair syntheses of DNA sequences and the breakdown of superfluous sequence ends is necessary. This process takes place in the recombination nodules in the synaptonemal complex, where all the necessary enzymes are concentrated.

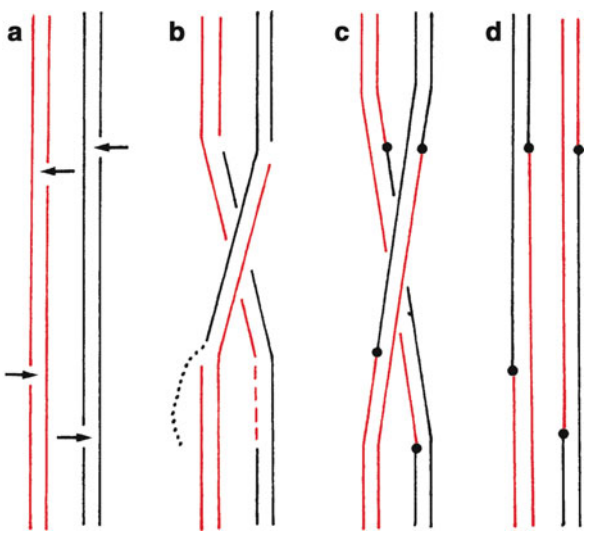

Fig. 2.41
Molecular processes in intrachromosomal recombination. (a) In the DNA double helices of two of the (in total four) paired nonsister chromatids, enzymatically generated single-strand bridges are induced at about the same height (*arrows;* helical structure of the DNA is not shown). (b) Crossing-over after alternate pairing, superfluous single strands (*dotted*) are broken down; absent ones (*striped*) are supplemented by repair synthesis. (c) Ligation of the free ends. (d) Crossing-over results in the novel combination of maternal and paternal genes

2.2.3.9 Syngamy

Syngamy is a type of cell fusion more precisely it is the fusion of two gametes of different sexes. It starts with **plasmogamy**, creating a binucleate cell. Plasmogamy is generally immediately followed by **karyogamy**, either by fusion of the nuclear envelopes of the two gamete nuclei (prenuclei) or by the breakup of the envelope and the arrangement of the maternal and paternal chromosomes on a shared spindle apparatus, meaning the first diploid mitosis can take place immediately. Syngamy and karyogamy can be both spatially and temporally separated, like in many ascomycetes and basidiomycetes. The two parts of the syngamy process are separated by a **dikaryophase**; the affected cells contain two nuclei.

There is a huge diversity of forms of syngamy in nature. In some cases, no specialized gametes are formed because any cells in the partners' bodies can fuse with each other (**somatogamy**, e.g., in *Spirogyra* and in the higher fungi). In other cases, the gametes are highly differentiated cells brought together by an unbelievable array of adaptations. See the detailed explanations for the individual systematic groups in Sect. 10.2.

2.2.4 Ribosomes

Ribosomes are ribonucleoprotein complexes on which protein biosynthesis takes place. Rapidly growing cells in meristems are especially rich in ribosomes.

Protein biosynthesis is based on the **translation** of polynucleotide sequences into polypeptide sequences (see Sect. 6.3.1.2). This process requires huge amounts of energy and information and thus needs functional units of the necessary size and diversity. In fact, the particle masses of eukaryotic cytoplasmic ribosomes are about 4 MDa (*Table 2.2*).

Prokaryote ribosomes are smaller than those of the eukaryotes. Ribosomes are identified according to their sedimentation behavior in the ultracentrifuge: 70S or 80S. They differ not only structurally but also functionally. Translation in 70S ribosomes is blocked by the antibiotics

Table 2.2

Some ribosome data

Property	Cytoplasmic ribosomes		Plastid ribosomes		*Escherichia coli* ribosomes	
Diameter (nm)	33		27		27	
Mass (kDa)	4,200		2,500		2,500	
Sedimentation	80S		70S		70S	
Protein fraction (% dry weight)	50		47		40	
Subunits	**60S**	**40S**	**50S**	**30S**	**50S**	**30S**
Number of ribosomal proteins	49	33	30	23	34	21
ribosomal RNAs	28S	18S	23S	16S	23S	16S
	5.8S		5S		5S	
	5S		4.5S			

All ribosomal RNAs and almost all ribosomal proteins are present only once in a single ribosome. Mitochondrial ribosomes can be, in part, quite differently developed in different organisms

chloramphenicol, streptomycin, lincomycin, and erythromycin, whereas the same concentrations of these antibiotics are ineffective against 80S ribosomes. Conversely, cycloheximide inhibits the action of 80S ribosomes. The organelle ribosomes of the plastids and mitochondria are, in many ways, more similar to the bacterial 70S ribosomes (*Fig. 2.42*) than to the eukaryotic 80S cytoplasmic ribosomes.

All ribosomes – prokaryotic ribosomes, organelle ribosomes, and eukaryotic cytoplasmic ribosomes – are composed of two unequally large subunits. These subunits are usually only bound together during translation, or more precisely, during the elongation of a newly forming polypeptide chain. As the completed polypeptide is released (termination), the ribosomal subunits separate from each other. The smaller can then bind with 5′-terminal sequences to new mRNA (initiation) and, after attaching to a larger subunit, can start elongation in the repetitive reaction sequence.

Both ribosome subunits are associates of many different, in part also basic, **ribosomal proteins** with various **rRNAs**: the 80S ribosome is made up of a 40S subunit (with18S rRNA and 33 proteins) and a 60S subunit (with 5S, 5.8S, and 28S rRNAs and 49 proteins), and the 70S ribosome is made up of a 30S subunit (with 16S rRNA and 21 proteins) and a 50S subunit (with 5S and 23S rRNAs and 32 proteins).

The molecular architecture of the ribosomal subunits, down to their atomic arrangements, was first determined for bacterial ribosomes. The interaction between mRNA and tRNAs takes place where the "head" of the small subunit and the "corona" of the large subunit oppose each other (*Fig. 2.42*). The growing peptide chain migrates from here through the large subunit and exits from the opposite blunt end of the subunit. In cytoplasmic ribosomes, about 40 amino acid residues of the growing polypeptide chain are protected within the ribosome and cannot be attacked by, e.g., peptidases/proteinases.

As well as mRNA (as an information carrier) and rRNAs (structural mediators and bonding partners), **tRNAs** are also involved in translation. They conduct activated amino acid residues to the ribosome and facilitate their assembly in the growing polypeptide chain. In the process, they pick the information encoded in the codons of mRNA using anticodons and by forming temporary base pairs.

The tRNAs are comparatively small molecules made up of about 80 nucleotides (about 25 kDa). Their sequence allows extensive intramolecular base pairing in which a characteristic tRNA cloverleaf structure is formed with four arms and three loops (*Fig. 2.43a*). The so-called acceptor arm has 3′ and 5′ ends and has no loops; the activated amino acid residue binds to the 3′ end. The anticodon for this amino acid, which can bind with a complementary mRNA triplet, lies opposite it. In fact, tRNA is not a two-dimensional structure but an L-shaped molecule. Acceptor ends and anticodon loops are oriented about 9 nm apart at each end of the L (*Fig. 2.43b*). The two side arms (with loops) of the cloverleaf are folded outward and contain recognition signals for those enzymes needed for each tRNA to load its specific amino acids. These enzymes, the **aminoacyl-tRNA synthetases**, are amazingly reliable and guarantee translation to a high standard

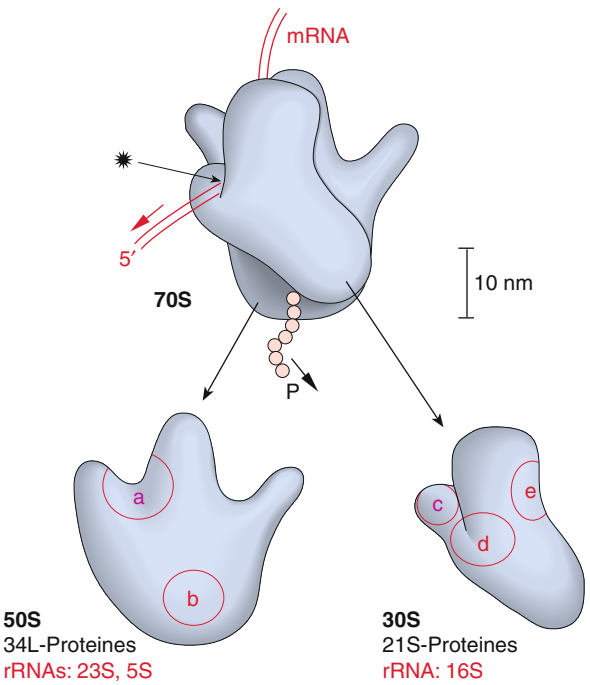

Fig. 2.42

Ribosome structure of 70S ribosome of *Escherichia coli*. Large and small subunits are paired in the active ribosome. The translation process occurs at the positions between the subunits marked by a *starred arrow*. The growing polypeptide chain (*P*) emerges at the lower end of the larger subunit. Functional positions on the subunits: *a* polypeptide synthesis (peptidyl transferase center); *b* exit of the polypeptide chain and membrane attachment; *c* mRNA attachment, codon–anticodon recognition; *d* transfer RNA (tRNA) attachment; *e* interaction with elongation factors. The ribosomal proteins of the large subunits are tagged with L1, L2, etc., and those of the small subunits are tagged with S1, S2, etc. Cytoplasmic ribosomes of eukaryotes (80S type) have similar outlines but are larger. *rRNA* ribosomal RNA

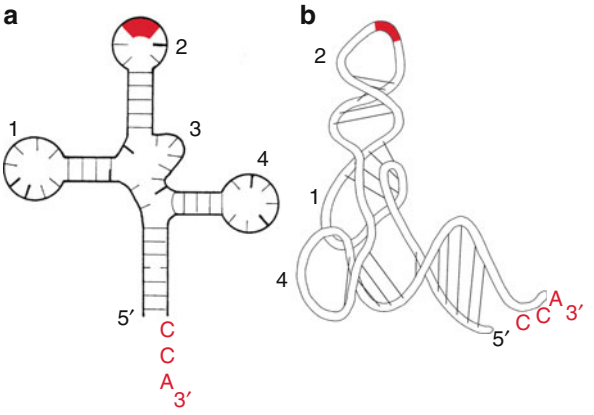

Fig. 2.43

Transfer RNA (tRNA). (a) Cloverleaf shape with four arms and three loops. *1* so-called T-Psi-C loop (ribothymidine–*ps*eudouridin–*c*ytidine; tRNA binds loosely with it to the 5S rRNA or 5.8S rRNA); *2* anticodon loop with anticodon (*red*); 3 variable loops, with different tRNAs of various sizes or completely lacking; *4* dihydrouridine loop. The preactivated amino acid is tacked onto the CCA sequence at the 3′ end. "Rare bases" are represented by *thick lines*. (b) Spatial model, "L form"

of precision, even compared with modern technology. The survival of cells and organisms would be impossible without this degree of accuracy.

The rRNAs and tRNAs occur in all life forms, from the smallest bacterium to the largest multicellular organisms, with a basically similar structure and with the same function. Their sequences have been extremely highly conserved during evolutionary history. They are thus especially reliable witnesses of evolution and enable the reconstruction of even quite ancient phylogenetic processes. An example is the special status and the great heterogeneity of archaea among the prokaryotes, clarified in great part as a result of rRNA sequence comparisons.

During translation, several to many ribosomes (monosomes) are strung together on an mRNA strand and form a **polysome** (*Fig. 2.44*). Polysomes are the actual translation organelles. They exist freely in the plasma and are spiral-shaped. On membranes, they form two-dimensional shapes, usually spirals (*Fig. 2.50b*). The membrane attachment is via the larger ribosomal subunit, near the exit for the growing polypeptide chain. These chains are often pushed through the membrane as they grow. This is how, e.g., secretory proteins and lysosomal enzymes get into the ER cisternae. In other cases, the nascent polypeptide chain is permanently anchored in the membrane by a series of at least 20 successively strung hydrophobic amino acid residues, becoming an integral membrane protein (see Sect. 2.2.5.1). Free polysomes primarily synthesize soluble proteins of the plasma cell compartments. They also translate mitochondrial and plastid proteins as well as all nuclear proteins and the characteristic peroxisome enzymes, which reach their destination later (after translation) (see Sect. 6.3.1.4).

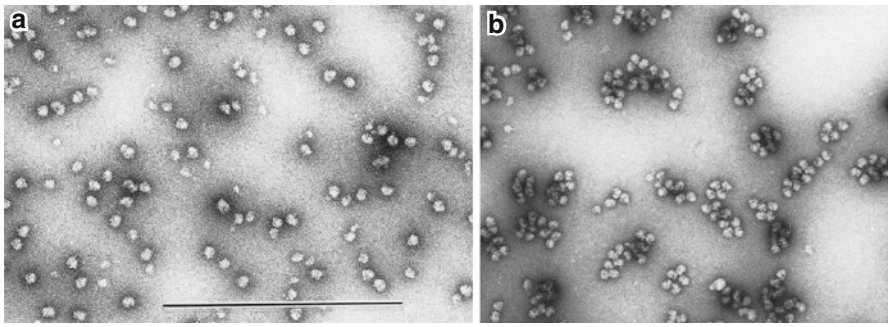

Fig. 2.44
Ribosomes and polysomes isolated from flower buds of *Narcissus pseudonarcissus*, negative contrast. (a) Monosomes. (b) Polysomes; ribosomes made up of two unequally sized subunits are partly visible. *Scale bar* 0.5 μm (Specimens from R. Junker; EM images from H. Falk)

2.2.5 Membranes

Biomembranes are 6–11-nm-thick, flat **lipoprotein structures** (see Sect. 1.5.2). They surround every single cell and also separate various compartments within the cells from each other. Two features enable them to perform this function particularly well: they are **selectively permeable** (see Sects. 2.2.5.3, 5.1.5), and they have no edges but entirely surround a compartment without a break. Despite being flat, they are not two-dimensional structures but are three-dimensional structures. Biomembranes are viscous: when artificially ripped open, they close-up immediately – a result of their hydrophobic property (see Sect. 1.5.1; this is why, e.g., it is possible to perform microinjections on living cells).

Membranes do not arise de novo in cells but are always derived from preexisting membranes. Membrane biogenesis occurs by surface growth of preexisting membranes that undergo integration of new molecules before separation of compartments by cytoses. The two most important building blocks of biomembranes are structural lipids and membrane proteins, which are both mainly synthesized in the ER. From there, vesicle streams carry them to the Golgi and vesicle membranes, as well as to the plasma membrane and to the outer envelopes of the plastids and mitochondria. The inner membranes of these organelles, which also significantly differ in terms of substance composition from other membranes, never come into direct contact with the other membranes of the cell.

2.2.5.1 Molecular Components

Artificially generated biomembrane films made of **structural lipids** (see Figs. 1.21, 1.22) are the same as biomembranes in terms of thickness, fluidity, and semipermeability, but they have no specific, active membrane transport. Also, the inner and outer surfaces of these artificial lipid films are identical, unlike biomembranes. These differences are due to the presence and absence of membrane proteins. It is the **membrane proteins** that enable the individual membranes in a cell to perform their various functions. The ratio of protein to lipid in a membrane is usually 3:2, but this can vary greatly. In protein-dominated membranes such as the inner membrane of mitochondria, the protein fraction is more than 70%, whereas in lipid-dominated membranes such as those of membranous chromoplasts (see Sect. 2.2.9.2), the protein fraction is under 20%.

There are two sorts of membrane proteins. **Peripheral** (extrinsic) **membrane proteins** sit on the surface of the lipid bilayer and are attached by electrostatic interactions to the polar particles of the membrane lipid; they do not come into contact with the apolar hydrocarbon chains of the lipids. This means they are easily dissociated from the biomembrane, e.g., by raising the ion concentration. Some peripheral membrane proteins are permanently anchored in the membrane by hydrocarbon chains covalently bonded with fatty acids or prenyl lipids. **Integral** (intrinsic) **membrane proteins** extend through the polarized inside of the lipid bilayer; they are transmembrane proteins. They can only be isolated from the lipid bilayer by destroying it, e.g., by applying detergents. These protein molecules are characterized by hydrophobic surface areas. Often these are α-helix regions of 20–25 amino acids with apolar side chains such as leucine, isoleucine, valine, and alanine (see Fig. 1.11). There are integral membrane proteins with several membrane channels and, accordingly, many hydrophobic α-helix domains. For example, bacteriorhodopsin has seven; ion channels have up to 24.

The integral membrane proteins are anchored in the lipid bilayer of the membrane because of the hydrophobic effect, and there are direct interactions between the proteins and the apolar hydrocarbon chains of the lipid molecule. Those domains of the transmembrane proteins that extend out from both sides of the membrane have hydrophilic surfaces. Many membrane proteins are glycosylated and bear covalently bonded sugar residues or oligosaccharide chains on the outside of the membrane.

2.2.5.2 The Fluid Mosaic Model

According to the fluid mosaic model, a typical biomembrane is a constantly changing mosaic of transmembrane proteins, with their hydrophobic domains integrated in a fluid-crystalline double film of structural lipids (Fig. 2.45). Although its fluidity allows the integral proteins to turn and shift sideways (lateral diffusion) within the plane of the membrane and the lipid molecules constantly change their position in the lipid film, the probability that the molecules flip (flip-flop) is almost completely excluded because of the hydrophobic effect. This means that a lipid molecule situated in the single lipid layer of a membrane double layer cannot switch to being in the other lipid layer, and the hydrophilic domains on both sides of the transmembrane domain of an integral membrane protein cannot switch position. Thus, biomembranes are asymmetrical; the outer and inner surfaces differ in their composition and properties.

During temperature changes, the fluid state of a cellular membrane is maintained by shifting the pattern of lipids. Augmented incorporation of sterol lipids and increased numbers of *cis* stereoisomer double bonds in the hydrocarbon chains of the lipid fatty acids promote fluidity. Organisms that live in colder environments have more unsaturated fatty acids integrated in their membrane lipids. Instead of the saturated double-bond-free stearic acid being involved in lipid synthesis, the unsaturated oleic, linoleic, linolenic, and arachidonic acids with one, two, three, and four double bonds, respectively, in an 18-carbon-atom fatty acid residue are used in lipid synthesis. The valuable linseed oil (with many double bonds) comes from cold growing regions.

Under the electron microscope, a transversely cut biomembrane appears as thin double lines (Fig. 2.46a, b), an expression of its bilayered construction. Integral membrane proteins are visible in freeze-fracture specimens as **inner membrane particles** (integral membrane protein; Figs. 2.66, 2.84, 2.93c).

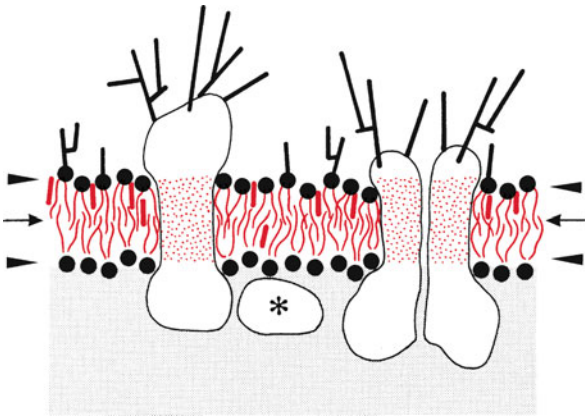

Fig. 2.45
Transverse section through a cell membrane according to the fluid mosaic model. The viscous lipid bilayer is pierced by integral membrane proteins (to the *right*, there is a dimer) whose extraplasmic domains bear unbranched and/or branched glycan chains. The glycolipid glycan chains also stand out from the extraplasmic sides of the membrane. Neither lipids nor proteins on the plasma side are glycosylated. Sterol lipids are stored in the nonpolar regions of the lipid bilayer (*red*); the transmembrane domains of the membrane proteins are also hydrophobic on the outer side. *Asterisk* peripheral membrane protein, *arrows* fracture zone from freeze fracture, *arrowheads* preferred deposition of contrast-generating osmium atoms that cause the trilaminar aspect of the transversely sectioned biomembrane under the EM (see Fig. 2.46a). All molecules involved are thermally active; exchange reactions occur continuously in the membrane plane and rotations occur continuously about axes perpendicular to the membrane plane. The spontaneous flipping (flip-flop) of lipid or protein molecules is practically impossible

2.2.5.3 Membranes as Compartment Boundaries

The existence of cells and cellular compartments would not be possible without the barrier effect of membranes. An essential task of a biomembrane is the inhibition of free diffusion and to create reaction spaces. On the other hand, cells and metabolically active compartments are open systems and as such depend on the exchange of certain substances with their surroundings. Special recognition sites and channels for particular ions and molecules serve these requirements. The enrichment of certain ions or molecules in the cells or compartments is often

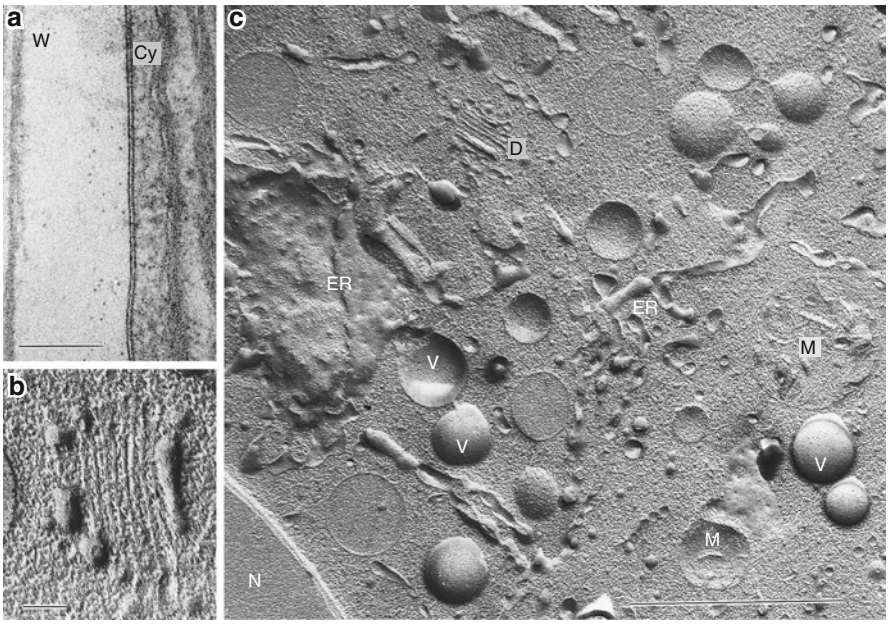

Fig. 2.46

Biomembranes in an EM. (a) Trilaminar cell membrane between the cell wall and the cytoplasm in the alga *Botrydium granulatum* after glutaraldehyde–OsO_4 fixation. (b) Trilaminar view of nonfixed Golgi membranes of a dictyosome after freeze fracture (transverse, embryonic cell of an onion root tip). (c) Partial view of a root meristem of the onion as a freeze-fracture specimen: numerous membranes in transverse section, as well as plane view with inner membrane particles whose number per unit area is characteristic of the membrane type. *Scale bars* (a, b) 0.1 μm, (c) 1 μm. *Cy* cytoplasm, *D* dictyosome, *ER* endoplasmic reticulum, *M* mitochondria, *N* nucleus, *V* vacuoles, *W* cell wall (a EM image from H. Falk; b, c specimens and EM images from V. Speth)

necessary. This is achieved by specific channels that function as pumps; i.e., energy-dependent **active** (**metabolic**) **transport**. The study of biomembrane permeability has shown that the barrier effect is basically due to the lipid bilayer, whereas specific, active membrane transport is performed by integral membrane proteins called **translocators** (permeases, carriers). Translocators recognize and bind to their permeates using steric fits (analogous to the specific formation of enzyme–substrate complexes; see Sect. 5.1.6) and are transported – with an accompanying conformation change – from one compartment to a neighboring compartment (see Sect. 5.1.5, Fig. 5.4).

Each compartment differs according to its particular composition (*Table 2.3*) and by an ionic, pH, and redox environment defined by the remaining compartments in the cell. If extremely high concentration differences (e.g., as a result of toxins, ionophores, or certain antibiotics) across compartment boundaries have to be leveled off too often, the cell dies.

Membrane potentials, which play an important role in a cell's life, also result from the different ion composition of neighboring compartments. The thinness of the lipid bilayer (4 nm) means that membrane potentials (on the order of 100 mV) can generate electrical fields with strengths of about 100,000 V cm^{-1}. This means the membrane potential is at the tolerance limit of the breakdown voltage for lipid bilayers.

Biomembranes are, however, not perfect diffusion barriers. Many lipophilic toxins, narcotics, and such substances are able to dissolve in the lipid bilayer and even concentrate there, and so the bilayer no longer presents a diffusion barrier. Even polar particles can pass through, when they are small enough (less than 70 Da). Membranes function as a filter with a median pore width of 0.3 nm. The pores are small, short-lived imperfections that are constantly generated as a result of the thermal movement of the lipid molecules in the fluid membranes. The comparatively high permeability for water is due in many cases to the presence of **aquaporins** that form 0.4-nm-wide transmembrane channels for water molecules (Fig. 5.23). When the channel is opened by phosphorylation of the aquaporin, neither ions nor metabolites

Table 2.3
Enzymes and compounds characteristic of cellular membranes and compartments

Cell component	Characteristic enzymes and compounds
Cell membrane	Cellulose synthase; Na^+/K^+ pump
Cytoplasm	Nitrate reductase; 80S ribosomes
Cell nucleus	Chromatin (linear nuclear DNA, histones, etc.); nuclear DNA and RNA polymerases
Plasma and nucleus	Actin, myosin, tubulin
Plastids	Starches and starch synthase; circular plastid DNA; plastid ribosomes (70S); nitrite reductase; in chloroplasts: ribulose bisphosphate carboxylase, chlorophylls, plastoquinone, plastid ATP synthase
Mitochondria	Fumarase, succinate dehydrogenase, cytochrome oxidase; ubiquinone; mitochondrial ATP synthase; circular mitochondrial DNA; mitochondrial ribosomes (70S)
Rough endoplasmic reticulum	Signal recognition particle receptor; ribophorins
Dictyosomes	Glycosyltransferases
Vacuoles/ lysosomes	Lysosomal acidic phosphatase, α-mannosidase; various storage substances, toxins, and pigments (proteins, sugars, acids; alkaloids, glycosides, calcium oxalate; flavonoids including chymochromes)
Oleosomes	Triacylglycerol

can pass through, but up to four billion water molecules can pass through per second.

The permeability behavior of the lipid bilayer in biomembranes is collectively described as the **lipid filter theory** (**lipoid-sieve theory**). It states that polar permeates can diffuse (sieve-like) through the hydrophilic pores of a membrane (depending on their size), whereas nonpolar permeates prefer to dissolve their way through a membrane. Apart from the parameters of particle size and lipophilicity, this passive permeation is not specific, and there are no specialized recognition structures for the various permeates. *Figure 2.47* demonstrates an informative experiment concerning the lipid filter theory, the ion trap (see also Fig. 6.37).

2.2.6 Cellular Membranes and Compartments

The different membrane systems of a cell are not directly connected to each other but can communicate with each other indirectly via vesicle streams, **membrane traffic** (**cytoses, endomembrane flows**). The fluidity of the biomembrane and the possibilities for shifting around even quite large membrane protein complexes within a membrane enable the functional diversification of spatially separated compartments and their membrane envelopes.

Cytosis requires the strict regulation of breakdown and fusion of compartments. Fusion of compartments is based on fusion of membranes. Biomembranes cannot spontaneously fuse, and therefore, the repulsion forces have to be overcome by special proteins. These proteins also ensure the specificity of the fusion of compartments (see Sects. 2.2.6.4, 2.2.6.5).

Most intracellular membranes (**endomembranes**) are connected to the cell membrane by cytotic processes and belong to a higher-order central membrane system. However, the mitochondrial membranes and the inner sheath membranes and thylakoids of the plastids do not belong to this system (these are discussed later; see Sects. 2.2.8.2, 2.2.9.1). Thus, the plant cell not only contains three permanently separated plasma types but also three independent membrane systems not connected by cytoses, each of which have their own characteristic lipid structure and protein features.

The large vacuoles and their membranes in the internal compartments of plant cells and fungi were first characterized in the nineteenth century as part of studies regarding osmosis. Nowadays, it is possible to isolate them intact (*Fig. 2.58*). The biochemical characterization of various cell compartments and their membranes by cell fractionation developed almost parallel with the electron microscope exploration of cell structures that revealed the central function of the ER and the nuclear envelope, as well as the Golgi dictyosomes and the various types of vesicles (*Box 2.1*). This enabled the identification of many structural and functional relationships. Even so, plant and fungal cells are not as easy to analyze as wall-less and vacuoleless mammalian cells.

2.2.6.1 The Cell Membrane

The **cell** or **plasma membrane** is thicker and denser than the other cellular membranes because of its high glycoprotein content. It generates and stabilizes the

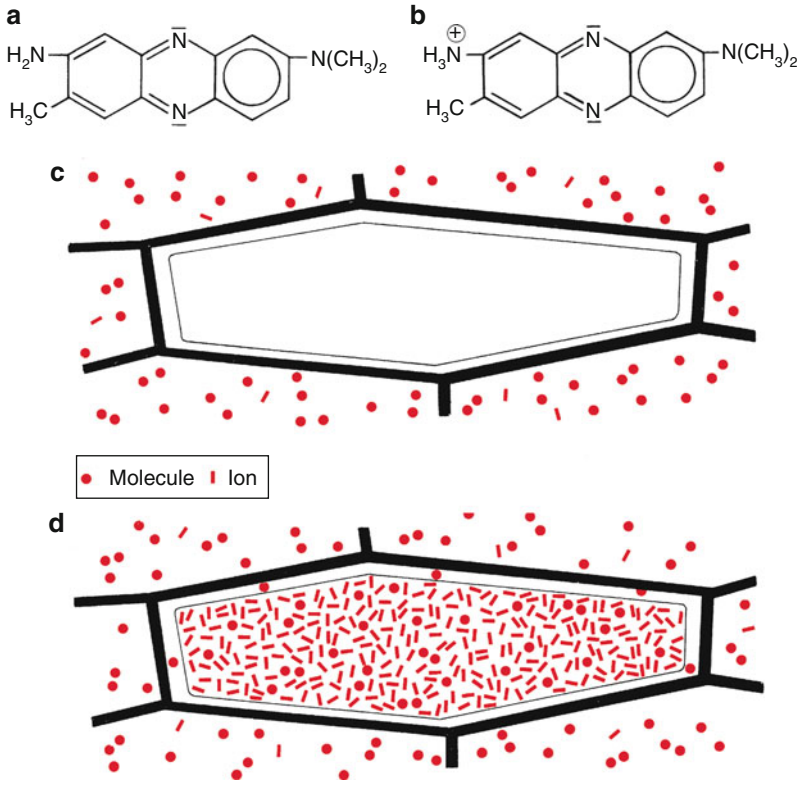

Fig. 2.47

Ion trap enrichment of neutral red in the vacuole of a plant cell. Neutral red occurs as a lipophilic molecule in alkaline solution (a); in acid, it occurs as a cation because it accumulates a proton (b). (c) Start point: living cell in diluted neutral red solution, pH 8 (dye molecules are represented by *dots*, dye cations are represented as *dashes*). (d) Final stage, dye molecules have permeated into the vacuoles (pH 5), which they are no longer able to leave because of their hydrophilic dye ions. Equilibrium is only reached once the concentration of neutral red molecules in the vacuole is equal to that in the external solution. This means that the neutral red ions have been enriched by more than 1,000 times in the vacuole

particular ionic settings in the cytoplasm using translocators driven by ATP to pump out protons and Ca^{2+} and Na^{2+} ions and pump in K^+ ions. The cell membrane is enlarged by protrusions or inclusions at those sites where intensive substance exchange takes place (see, e.g., Fig. 3.27).

Wall-less **protoplasts** are easily created by digesting the cell wall using pectinases and cellulases (*Fig. 2.48*). They are completely viable when osmotically stabilized. Protoplasts can be used to perform **cell fusions**, e.g., using poly (ethylene glycol) or electrical surges (*Fig. 2.49*). Accordingly, **cell hybrids** (**cybrids**) can also be artificially created between cells of completely disparate systematic origins that would never arise in nature.

The cell membrane is pressed against the inner side of the cell wall by turgor. In certain places, it is even more closely tied to the cell wall by specific chemical interactions. These sites are where cellulose microfibrils are manufactured (see Sect. 2.2.7.2). On the other hand, antibodies have been used to show that integral membrane proteins (integrins) interact with cell wall components and can form ligands (as in the extraplasmic matrix in animal cells).

2.2.6.2 The Endoplasmic Reticulum

The ER occurs in two structurally and functionally different forms: **rough ER** and **smooth ER**. The rough form, coated in polysomes, occurs as extensive flat cisternae that are characterized by the ability to rapidly change their conformation (*Figs. 2.50*, *2.51*). Conversely, the ribosome-free, smooth ER often forms a network of branched tubules (*Fig. 2.52*).

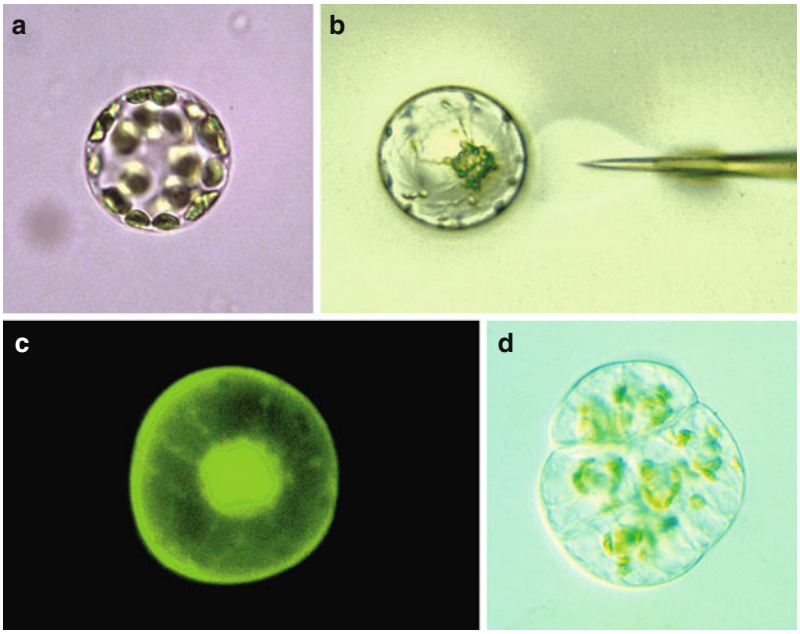

Fig. 2.48

Protoplasts artificially created by enzymatic digestion of the cell wall. (a) Protoplast from the mesophyll tissue (with chloroplasts) of *Nicotiana tabacum* after rounding off of the protoplasts in 0.6 M sorbitol (×200). (b, c) Microinjection into a protoplast of *Nicotiana tabaccum* (×200); (b) before and (c) after the injection of dextran–fluorescein isothiocyanate into the protoplast cytoplasm; visibly colored cytoplasm (c fluorescence microscopy). (c) Cell clone from divided protoplast in cell culture (regeneration)

Huge amounts of protein synthesis take place on the **rough ER**. The proteins formed by the membrane-bound polysomes are either integral membrane proteins or proteins that are destined to be stored in nonplasmatic compartments such as vacuoles or to be transported out of the cell (secretion proteins, export proteins, e.g., cell-wall proteins or the wall-destroying exoenzymes of parasitic fungi). The rough ER membranes are the only ones that have receptors for cytoplasmic ribosomes and are able to bind polysomes on their plasma side. For details on the translocation of proteins synthesized by ER-bound ribosomes, see Sect. 6.3.1.4.

The functions of the **smooth ER** are more diverse, it is most notably involved in lipid, flavonoid, and terpenoid synthesis (see Sects. 5.15.1, 5.15.2). The formation of fatty acids in plant cells occurs mainly in the plastids (in contrast to animal cells). The conversion of the initially saturated fatty acids into unsaturated fatty acids and the incorporation of newly formed lipids in the membranes is, like in all eukaryotic cells, also a function of the smooth ER. This assembly only takes place on the cytoplasmic side of the ER membranes, which means that the newly synthesized lipid molecules only enter the membrane layer abutting the plasma side. However, these membranes contain a special protein, **flippase**, which catalyzes the otherwise almost impossible flip-flop action of lipid molecules from the plasmic to the extraplasmic membrane layers.

2.2.6.3 Dictyosomes and the Golgi Apparatus

The dictyosomes are where (mainly) integral membrane proteins and secretory proteins are modified and sorted, before being transported by secretion vesicles (**Golgi vesicles**) out of the cell or stored in vacuoles. Dictyosomes are the elements of the Golgi apparatus. Small cells often have just a single dictyosome. Larger cells have numerous, sometimes over 1,000, dictyosomes, usually dispersed throughout the cytoplasm (unlike in many animal cells).

A typical **dictyosome** is constructed from a stack of **Golgi cisternae**. It is always found next to an ER cisterna or a nuclear envelope, oriented parallel to it

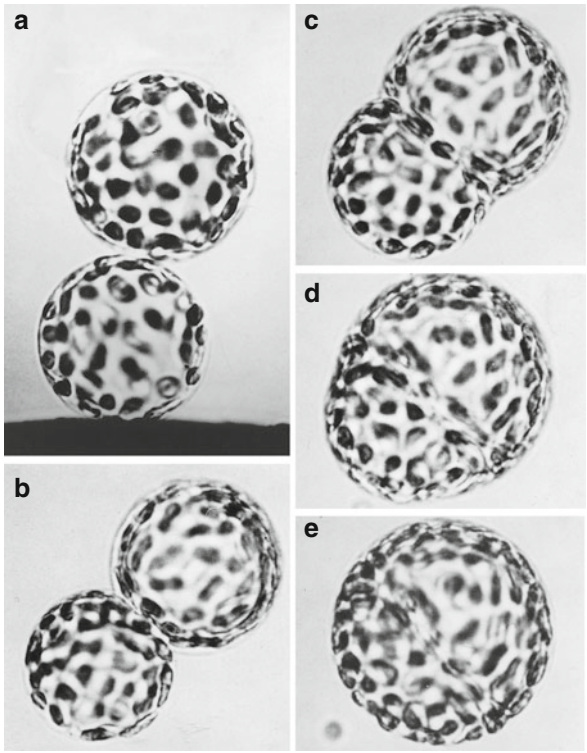

Fig. 2.49
Electrofusion of protoplasts of the foliose moss *Funaria hygrometrica* (×640). Two protoplasts touching each other at an electrode (a) are fused by an electric jolt (electric field strength 1 kV cm for 1.70 μs). (b–e) A new moss plant can grow from a hybrid cell formed this way in just a few weeks (Specimens and microimages from A. Mejia, G. Spangenberg, H.-U. Koop, and M. Bopp)

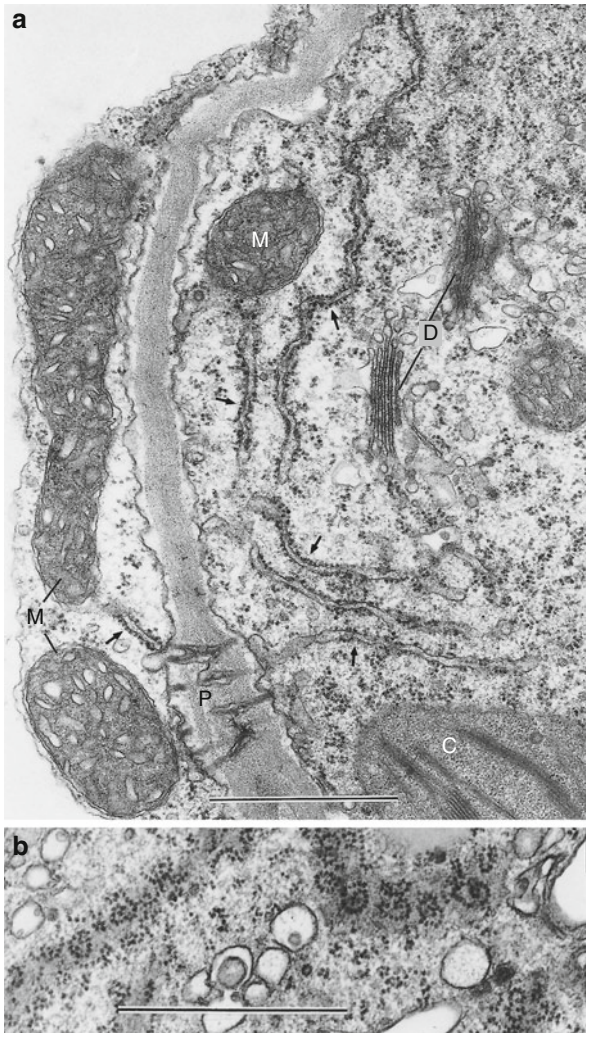

Fig. 2.50
Ribosome-studded endoplasmic reticulum. (a) Cisternae of a leaf cell of the green bean in transverse section (*arrow*), next to mitochondria (*M*), dictyosomes (*D*), and chloroplast (*C*); plasmodesmata (*P*) in a primary pit field of the cell wall. (b) Flat section of rough endoplasmic reticulum cisternae with spiral polysomes in a pollen tube of tobacco *Nicotiana tabacum*. *Scale bars* 1 μm (EM images from a H. Falk and b U. Kristen)

(*Figs. 2.53b*, *2.55*). The dictyosome side abutting the ER is termed the ***cis*** side, the ***trans*** side being the side facing away from the ER. New Golgi cisternae are formed on the *cis* side as vesicles flow together, whereas on the *trans* side, Golgi vesicles break away. In many cases, the marginal zones of the distal Golgi cisternae are formed reticulately (***trans*-Golgi network**). These so-called hypertrophic dictyosomes do not release single vesicles, but instead, whole cisternae become inflated and migrate to the cell surface (*Fig. 2.54d*).

Dictyosomes are not permanent structures. They can be formed by the ER on demand. The **structure of a dictyosome** differs from organism to organism and even from cell to cell within a multicellular organism. In some primitive fungi, and also in the cells of dried seeds, the dictyosomes are replaced by collections of small membrane vesicles or tubules. A plant cell dictyosome will typically have between four and ten Golgi cisternae, but this can increase to over 30 in protists.

Golgi cisternae are typically the sites of **oligosaccharide** and **polysaccharide synthesis**. Particular glycosyl transferases (e.g., galactosyl transferase carries galactose units to growing glycan chains) are characteristic enzymes of the Golgi apparatus.

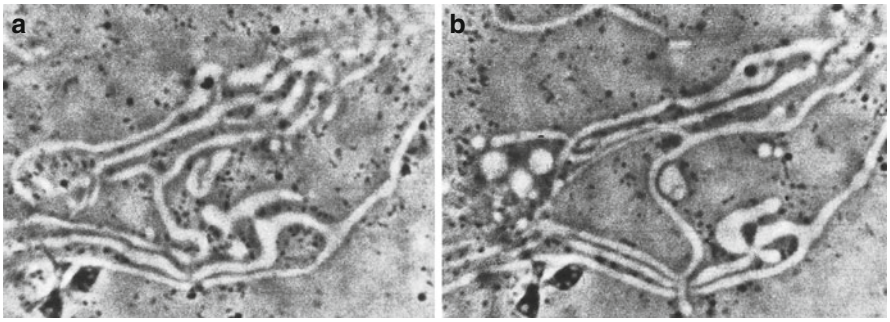

Fig. 2.51
(a) Endoplasmic reticulum in a living tissue culture of tobacco (×940). (b) Taken 10.5 s after (a). In (a) and (b), there are, next to the endoplasmic reticulum, also oleosomes, mitochondria, and (*bottom left*) plastids (Phase contrast images from W. Url)

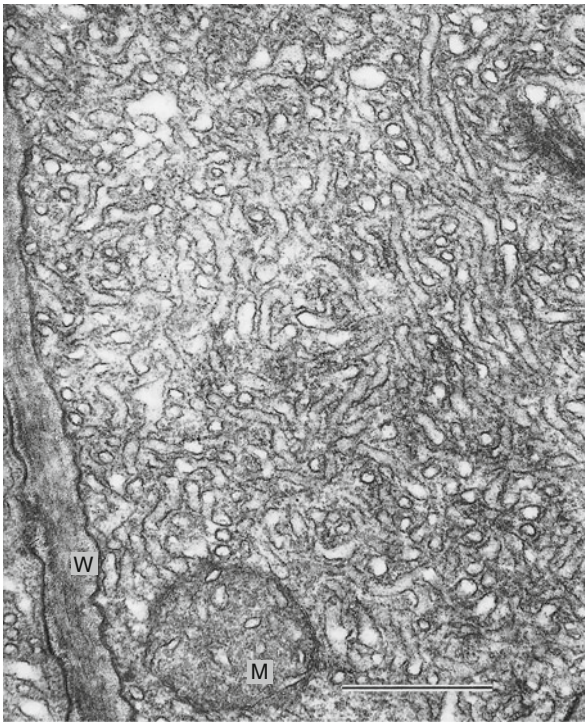

Fig. 2.52
Smooth endoplasmic reticulum in an oil gland cell of the burr *Arctium lappa* with numerous transverse and lengthwise sections through the twisted and branched endoplasmic reticulum tubules. *Scale bar* 0.5 μm, *M* mitochondria, *W* cell wall (EM image from E. Schnepf)

They are involved in, e.g., the building of polysaccharides for the cell wall matrix (see Sect. 5.16.1.1; for the synthesis of storage polysaccharides such as starch and glycogen, there are specific cytoplasmic or plastid or mitochondrial enzyme systems; see Sect. 5.16.1.2). The Golgi cisternae are also where the extraplasmic domains of integral membrane proteins become glycosylated – a process that starts in the rough ER but is completed in the Golgi apparatus. All export proteins and integral proteins of the plasma membrane are glycoproteins.

Even the secretions differ greatly. One extreme is seen in the **structured secretions**; self-organizing processes in the Golgi cisternae or vesicles form characteristic structures. Well-known examples include cell-wall scales (Figs. 10.82, 10.83g), less well known are the extrusomes (ejectosomes or trichocysts) of the single-celled cryptophytes and dinophytes – explosively catapulted, sometimes poisonous missiles for defense against predators or to paralyze prey – as well as the mastigonemes of shimmer flagella (Figs. 10.21a–c, 10.89f). In addition, there are the especially watery polysaccharide slime secretions. A peculiar case of Golgi secretion is the **active secretion of water**. All freshwater protists without cell walls are, to a certain extent, unstable as they take up water osmotically, but they are then unable to compensate for the rise in internal pressure with an exoskeleton. These organisms possess systems whereby they are able to actively secrete water, usually via pulsating (**contractile**) **vacuoles**. These enlarge as they suck up water, either mechanically or osmotically, from the surrounding plasma (diastole) and periodically contract, squirting it out through a temporarily opened channel (systole). In the unicellular alga *Vacuolaria*, this function is taken over by numerous dictyosomes. They form a dense perinuclear Golgi apparatus immediately around the nuclear envelope. Golgi vesicles constantly detach in great number. They contain a water-rich slime and coalesce in rapid succession to form ever-larger secretory vacuoles before being expelled by exocytosis (*Fig. 2.54*).

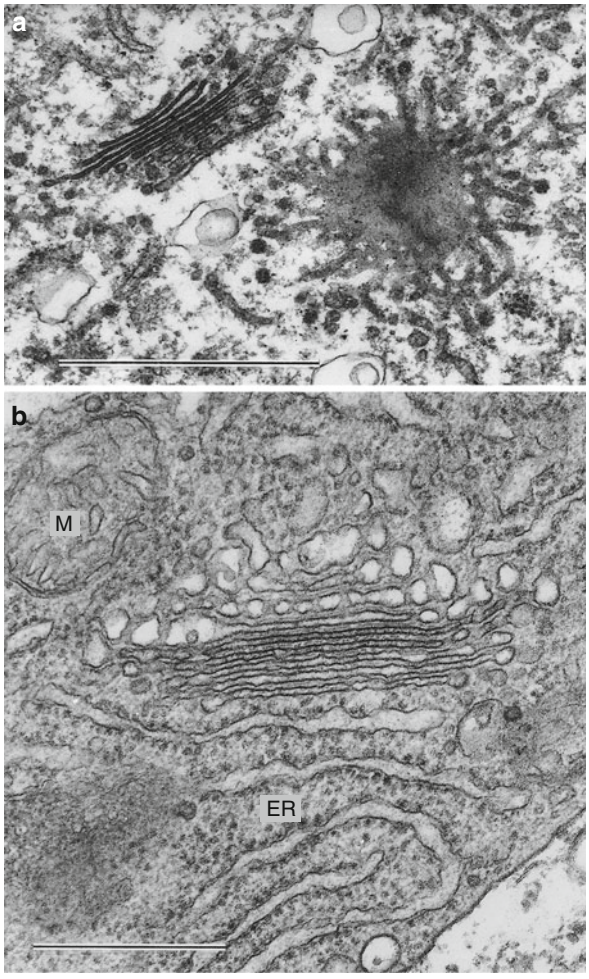

Fig. 2.53
Dictyosomes. (a) A planar and transverse section of a dictyosome from the ligule cell of Merlin's grass *Isoetes lacustris*; tubular reticulate periphery of the Golgi cisternae and multiple small vesicles (*scale bar* 1 μm). (b) Transverse section of a dictyosome in a gland cell of *Veronica beccabunga*; *cis*-side down, oriented to the rough endoplasmic reticulum; on the *trans* side delicate Golgi filaments are visible between the cisternae; outer cisternae on the *trans* side are dilated and fenestrated (*trans*-Golgi network) (*scale bar* 0.5 μm). *ER* endoplasmic reticulum, *M* mitochondrion (EM images from a U. Kristen; b J. Lockhausen and U. Kristen)

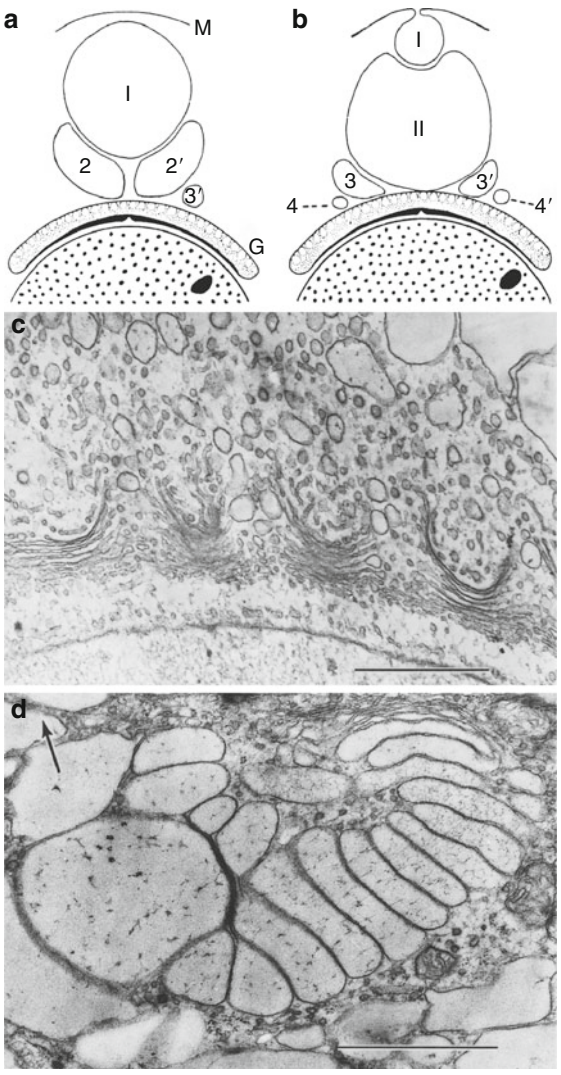

Fig. 2.54
Water secretion by the Golgi apparatus. (a–c) *Vacuolaria virescens*. (a, b) The formation and exocytosis of water-rich Golgi vesicles (vacuoles) in successive stages. The complex Golgi apparatus (*fine dots*), composed of about 50 dictyosomes abuts the nucleus (*large dots*). *Roman numerals* indicate the larger "pulsating vacuoles" that have arisen from the fusion of smaller vacuoles (*Arabic numerals*). Over 30 min, the amount of water secreted is the same as the cell volume. (c) Four dictyosomes of the Golgi apparatus and the Golgi vacuoles that increase in size toward the outer (*upper*) edge. (d) In the similarly unicellular alga *Glaucocystis geitleri*, whole Golgi cisternae inflate by water absorption and empty rhythmically outward in the direction of the *arrow*. (c, d) *Scale bars* 1 μm. *G* Golgi apparatus, *M* cell membrane (a, b After R. Poisson and A. Hollande; c, d EM images from E. Schnepf and W. Koch)

2.2.6.4 Membrane Traffic, Exocytosis, and Endocytosis

In contrast to membrane transport (relocation of substances through the biomembranes), membrane traffic (**vesicular transport**) refers to the transport of entire

compartments. Membrane traffic allows small partial compartments to detach from larger compartments, to be then directionally moved in the cell by using the cytoskeleton and motor proteins and also to merge with other compartments. An overview of these processes (**cytoses**) is given in *Fig. 2.55* (see also Fig. 6.16). Thus, secretory vesicles detach from the *trans*-Golgi network, migrate to the cell membrane, coalesce with it, and discharge their contents to the outside. The vesicle membrane becomes part of the plasma membrane. This type of cellular secretion is called **exocytosis**.

The detachment of secretory vesicles causes the dictyosome to lose membrane material. New membrane material has to be imported from the ER because the dictyosomes synthesize neither lipids nor proteins. This is performed by **transit vesicles** in precisely defined quantities, so the dictyosome appears unchanged despite the constant flux of material. It is a dynamic structure that maintains a fluid equilibrium and has a structural and functional polarity: new Golgi cisternae are formed from transit vesicles on the ER-oriented proximal, *cis* (formation) side, whereas on the distal (*trans*, secretion) side, the Golgi membranes are lost because of the detachment of secretory vesicles. Thus, Golgi membranes migrate, together with their secretion precursors, through the stack of cisternae from *cis* to *trans*, regardless of whether as they do this as entire cisternae or via the vesicle flows on the edge of the dictyosomes. In the process, the cisternae lose height and the membrane gains thickness. Membrane-bound enzyme activities can be proximal or distal. The stepwise relocation of oligosaccharide and polysaccharide chains means the glycan fraction of the prosecretion increases and at the same time the inside of the cisterna becomes acidified. The sugar residue sequences in the oligosaccharide chains of glycoproteins are determined by successive temporal and spatial building steps along an assembly line.

When folds in the cell membrane at the cell surface form vesicles that are then pushed into the cell, the process is called **endocytosis**. Formally, it represents a reversal of the last exocytosis step. In this way, e.g., macromolecules bound to the outer surface of the cell membrane by specific receptors can be transported by **coated vesicles** to endosomes and lysosomes for digestion. Many multicellular organisms and most animal cells can take up microscopic nutritive particles via endocytosis (**phagocytosis**; *Fig. 2.55*). Endocytosis has also been detected in plant cells, although phagocytosis is precluded

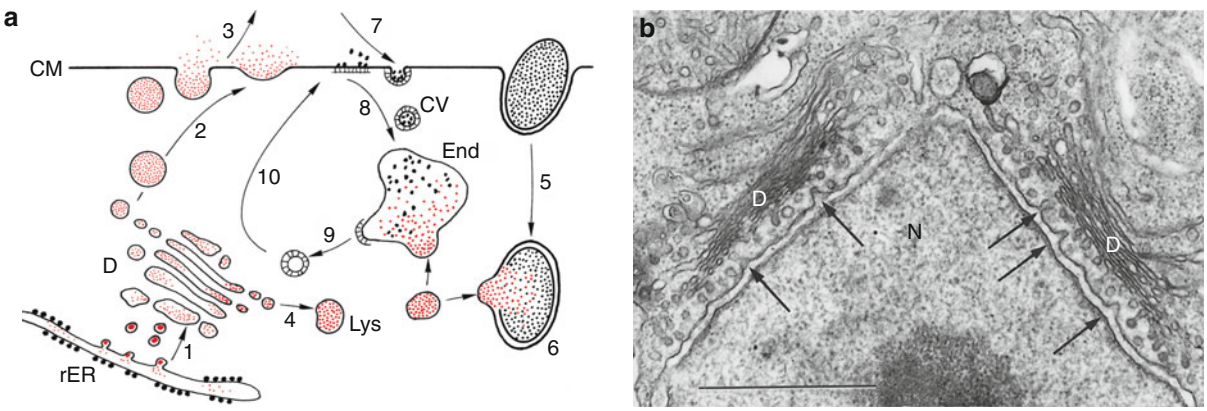

Fig. 2.55

Membrane flow, exocytosis, and endocytosis. (a) Proteins synthesized at the rough endoplasmic reticulum reach the dictyosomes (*D*) by transit vesicles (*1*). There they are modified by glycosylation and either transported by Golgi vesicles to the cell membrane (*2*) and exocytosed (*3*) or packed in primary lysosomes (*4*). Larger particles (*5*) that have been taken up by phagocytosis (*5*) are broken down by enzymes in digestive vacuoles (*6*). Smaller particles, e.g., useful macromolecules, are adsorbed by specific receptors in the cell membrane and transferred to the endosomes by coated vesicles (*8*), in whose acidic environment they disengage from the receptors and are hydrolyzed. The receptors are recycled, initially via cell membranes to dictyosomes (*9*) and then back to the cell surface (*10*). (b) Vesicle transport from the rough endoplasmic reticulum (represented here by the nuclear envelope of the alga *Botrydium granulatum*) to neighboring dictyosomes. *Arrows* pinching off of transit vesicles, *scale bar* 1 μm, *CM* cell membrane, *CV* coated vesicles, *D* dictyosome, *End* endosome, *Lys* lysosomes, *N* nucleus, *rER* rough endoplasmic reticulum (b EM image from H. Falk)

in these cells and cell walls because of their osmotrophy. Coated vesicles are found frequently in plant cells, especially around the cell membrane and surrounding dictyosomes (Fig. 2.56d). They are involved in the recycling of membranes and receptors or the intracellular shifting of membranes and substances (**intracytosis**).

2.2.6.5 Coated Vesicles

Coated vesicles (Fig. 2.56), with diameters of about 0.1 µm, are among the smallest compartments of a cell. They have a membrane skeleton on their plasma side – the coat. According to the coat proteins, there are two sorts of coated vesicles: clathrin vesicles and coat protein vesicles. **Clathrin vesicles** are seen during endocytosis and vesicle traffic between dictyosomes and lysosomes or vacuoles, whereas coat protein vesicles are principally seen in exocytosis and intracytosis. Both types of coated vesicles are short-lived and experience a constant turnover.

The honeycomb cage structure that the structural protein **clathrin** (Greek *clathron*, cage) forms around the clathrin vesicles is constructed from clathrin trimers, so-called triskelions (Fig. 2.56b). Construction and destruction are controlled by associated proteins, and the disassembly is controlled by, e.g., a special ATPase. The formation of clathrin vesicles by endocytosis is triggered by the laying down of a honeycomb of clathrin on the inner side of the cell membrane. This process involves assembly proteins that are related to the functionally analogous proteins in mammalian cells (adaptins). The affected regions of the plasma membrane are called coated membranes or, after sinking into the cytoplasm, coated pits. In plants cells, over 7% of the cell membrane surface can be occupied by clathrin polygons. The formation of coated pits and their binary fission as clathrin vesicles require energy to be exerted against turgor (compare this with local depression of a fully inflated bicycle tire inner tube). Accordingly, endocytosis in plant cells tends to be observed where turgor is low (root hairs, endosperm cells, artificially created protoplasts). The developing clathrin coat is associated with **dynamin**, a GTPase that meets the energy requirements by splitting GTP. **Coat protein vesicles** have a coat complex composed of several proteins, unrelated to clathrin, called **coatomers**. The important steps in vesicle transport have mostly been identified in yeast cells (on which genetic analyses are particularly easy to perform) and in mammalian nerve cells (in which the mass release of neurotransmitter vesicles is controlled by electrical impulses).

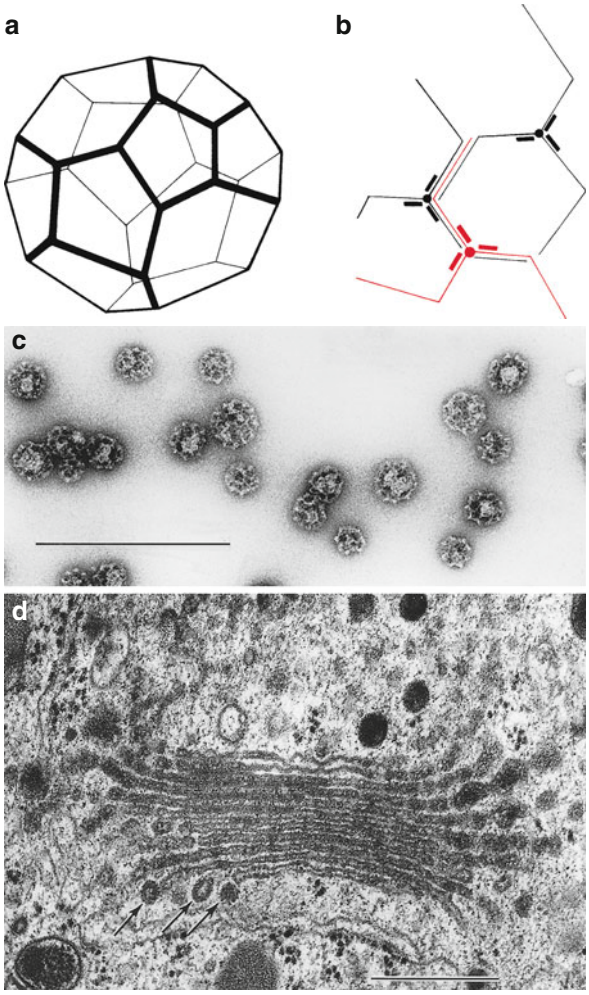

Fig. 2.56
Coated vesicles and clathrin. (a) A clathrin vesicle. (b) Three triskelions (one in *red*) as the building blocks for the pentagonal and hexagonal lattice. Each triskelion consists of three heavy chains (each 180 kDa, 50% α-helix, bent arms) and three light ones (each 35 kDa); four heavy chains run along every edge of the clathrin lattice and the light chains sit in the corners. (c) Coated vesicles isolated from the hypocotyl of zucchini (a cultivar of pumpkin *Cucurbita pepo*) in negative contrast. (d) Coated vesicles (*arrows*) on a dictyosome of the alga *Micrasterias* (note also the one-sided ribosome complement of the endoplasmic reticulum cisternae opposite the *cis* side of the dictyosome). (c, d) *Scale bars* 0.5 µm (c Specimen and EM image from D.G. Robinson; d EM image from O. Kiermayer)

These steps include the vesicle budding on the donor membranes, their docking on the target membrane, and their fusion with the latter. To form vesicles, coatomers from the cytoplasm are built into

coats in the affected membrane regions of the ER or the dictyosomes. This requires the activation of a small G protein that contains GDP and which then exchanges this for GTP. This process, which triggers vesicle budding, can be blocked using the fungal toxin brefeldin. The docking of the vesicle on the target membrane is only possible after the coat has been burst. This process is performed by the splitting of GTP in the G protein. Additionally, specific recognition structures are also necessary in order to eliminate the possibility of miscarriages in the vesicle transport. A number of such structures have been characterized. They are summarized under the acronym **SNARE** – v-SNARE (on the vesicle) and t-SNARE (on the target membrane).

2.2.6.6 Peroxisomes and Glycoxysomes

At the start of the electron microscope era, the term "**microbodies**" was coined, referring to all dense vesicles between 0.3 and 1.5 µm that contribute to special metabolic processes and therefore contain certain enzymes in high concentrations. The function of microbodies differs from cell (tissue) to cell (tissue) but always features oxidative chemical changes, usually in terms of substance breakdown. These reactions generate the cell toxin hydrogen peroxide (H_2O_2), which is then broken down by catalase into water and oxygen. Catalase is the characteristic enzyme for microbodies, collectively referred to as **peroxisomes**. In plants, the peroxisomes of photosynthetically active cells (**leaf peroxisomes**; *Fig. 2.57*) are known as the organelles of photorespiration (see Sect. 5.5.6), and the peroxisomes of oil-storing seeds, the **glycoxysomes**, are important in the mobilization of fat reserves (see Sect. 5.11). In both cases, the metabolic relationships in the living cell are topologically evident in the close congregation of the peroxisomes with the plastids and mitochondria or oleosomes.

Peroxisomes are only produced by peroxisomes even though, unlike mitochondria and plastids, they do not have any nucleic acids. All characteristic enzymes are synthesized on the free polysomes of the cytoplasm and only later, when a transit peptide partial sequence splits, are they relocated to the microbodies. This is reminiscent of the processes found in mitochondria and plastids (see Sects. 2.2.8.2, 2.2.9).

2.2.6.7 Vacuoles and Tonoplast

Cell-sap-filled vacuoles, especially the large **central vacuoles** of tissue cells, are characteristic of plant cells. The volume of all vacuoles together in a cell accounts for nearly

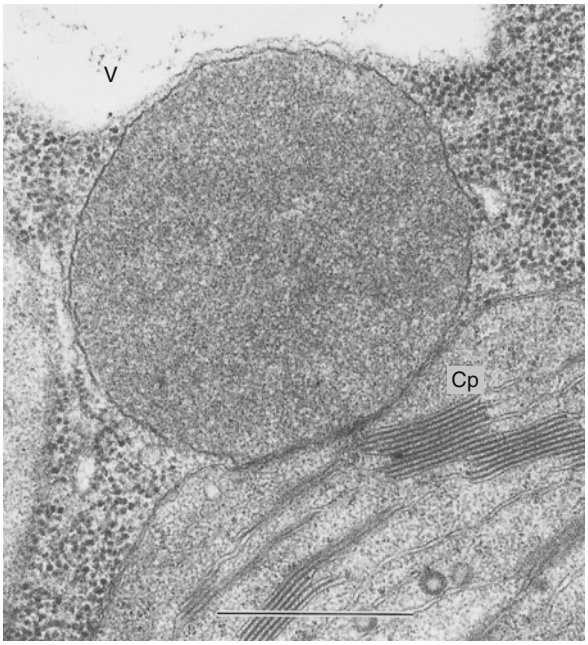

Fig. 2.57
Leaf peroxisomes of spinach, tightly aggregated on a chloroplast (with grana). Numerous ribosomes in the cytoplasm. *Scale bar* 0.5 µm, *Cp* chloroplast, *V* vacuole (EM image from H. Falk)

20% of the cell volume in primary meristems, but can reach proportions of over 90% (see *Figs. 2.2a, c*, *2.48a–c*, *2.58*). Vacuoles are nonplasmatic compartments; their contents have a pH of about 5.5 or sometimes lower. The vacuoles are separated from the weakly alkaline cytoplasm by the **tonoplast membrane** – usually simply referred to as the tonoplast.

Under normal conditions, the molar concentration of **cell sap** is far greater than that of the liquids in the cell walls, which is practically pure, demineralized water. The cell sap is hypotonic and sucks up water through the plasma membrane and the tonoplast (**osmosis**; see Sect. 5.3.2.1). The resulting hydrostatic pressure – **turgor** – tensions the cell wall and is countered by wall pressure. As a liquid, the cell sap is not compressible, and the stability of herbaceous, nonwoody plant parts is based on the antagonism between turgor and wall pressure. When the cell walls are breached or become weakened, e.g., like in the glandular tissues of plants, turgor can help the expulsion of secretions.

If the external medium of a cell is experimentally made hypertonic with respect to the cell sap, then the vacuole

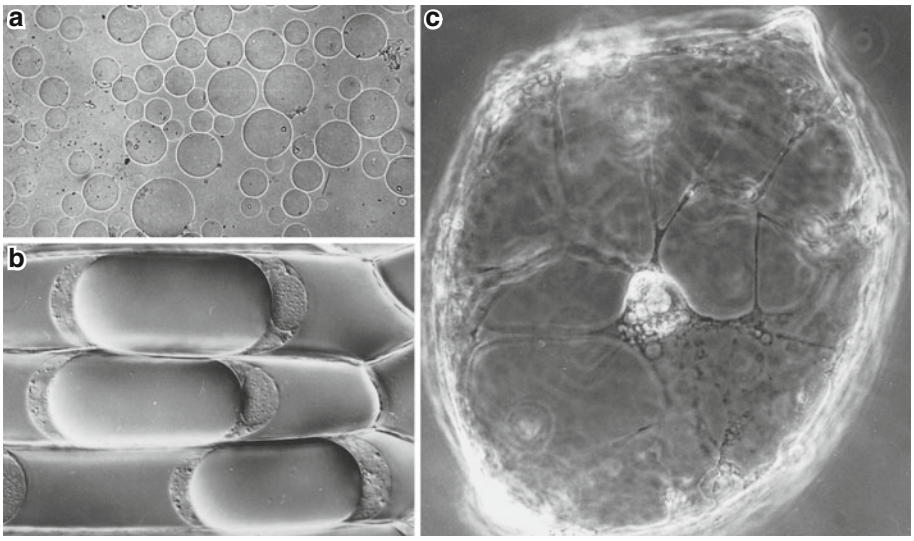

Fig. 2.58
Vacuoles. (a) Isolated from protoplasts of the storage root parenchyma of the sugar beet *Beta vulgaris* ssp. *altissima* (×320). (b) In plasmolyzed cells of the bulb epidermis of onion *Allium cepa*, plasma tubes are swollen because of the plasmolyte (1 M KSCN) (×210). (c) Pulp of the snowberry *Symphoricarpos albus*. The cell nucleus is anchored in the middle of the large central vacuole by plasma threads rich in actin filaments (×320) (a Specimen and image from J. Willenbrink; b interference contrast image from H. Falk; c phase contrast image from W. Url)

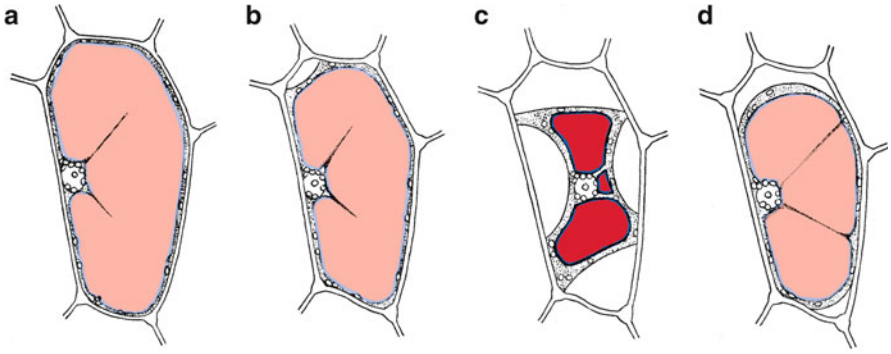

Fig. 2.59
Cells from the lower leaf epidermis of *Rhoeo discolor*. (a) In water. (b) Start of plasmolysis in 0.5 M KNO_3. (c) Plasmolysis completed, cell sap concentrated. (d) Deplasmolysis after placement in water (After W. Schumacher)

loses water until the molar concentration of all nonpermeable components of the cell sap has reached equilibrium with the external medium. The volume minimization of the vacuole initially relaxes the cell wall, and eventually the protoplast detaches from it: **plasmolysis** (*Figs. 2.58b*, *2.59*). But there are wall partitions that do not detach from the cell membrane even during plasmolysis (regions of negative plasmolysis, e.g., the Casparian strips in endodermises; see Sect. 3.2.2.4). In these places, the glycan chains of integral membrane proteins are firmly anchored in the cell wall.

W. Pfeffer demonstrated the semipermeability or selective permeability of membranes in living cells by using plasmolysis over 120 years ago. Around 1900, E. Overton developed the first ideas about the chemical and molecular properties of biomembranes.

Vacuoles are often **storage compartments**. The substances dissolved in the cell sap tend to be mainly organic

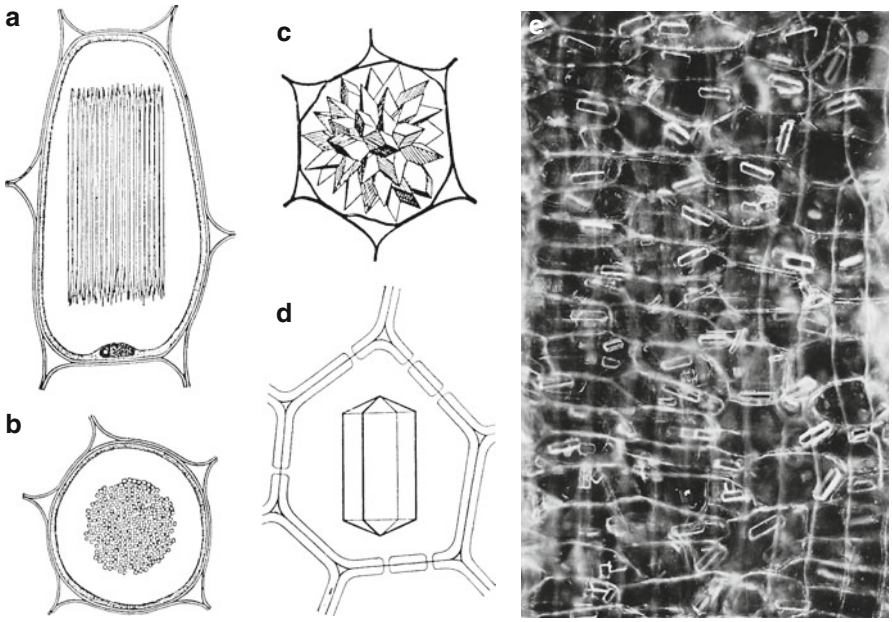

Fig. 2.60
Various forms of calcium oxalate crystals. (a, b) Raphids (bundle of crystals, monohydrate) in *Impatiens*, lengthways and transverse (×200). (c) Gland, monohydrate (*Opuntia*, ×200). (d) Tetragonal solitary crystal in a leaf epidermis cell of *Vanilla* (dihydrate, ×150). (e) Oxalate styloids in dried brown involucral bracts of the onion *Allium cepa* (dihydrate; dark-field image ×65) (a–d After D. von Denffer)

metabolites such as sugar and organic acids (malic acid, citric acid, oxalic acid, amino acids) as well as inorganic ions (K^+, Cl^-, Na^+). The vacuoles often serve as storage places for temporary excesses of metabolites (e.g., saccharose accumulation in vacuoles, which is especially pronounced in sugarcane and sugar beet; nocturnal dumping of malic acid or malate in Crassulacean acid metabolism plants; see Sect. 5.5.9). In the vacuoles of many cells, there are various forms of crystals of insoluble calcium oxalate (*Fig. 2.60*), which allows the deposition of excess calcium.

Even more conspicuous is the constant removal of compounds from the cytoplasm where synthesis takes place and their concentration in the vacuoles. The heterogeneity of these secondary metabolites is enormous. Most originate from the **secondary metabolism** of plants (see Sect. 5.1.5). A significant proportion of these compounds referred to as "**plant**" **or** "**natural**" **substances** are of, e.g., pharmaceutical importance or enable the use of the relevant plants for harvesting aromatic, luxury, or medicinal products.

In terms of human nutrition, some particularly important forms of substance storage in vacuoles can be found in many seeds, especially in those of the legumes and cereal grains. Seeds are particularly suited to transport and storage because of their low water content and their durability. At seed maturity, protein storage vacuoles or **aleurone grains** (Greek *áleuron*, wheat flour) develop in the peripheral cells of cereal grains and in the cotyledons of legumes (peas, beans, lentils, etc.) (*Fig. 2.61*). The storage proteins are synthesized on the rough ER. The aleurone grains arise either directly on the inflated rough ER cisternae or by the transfer of dictyosomes via the flowing together of Golgi vesicles (*Fig. 2.62*).

The storage proteins are frequently polymeric complexes with high particle masses (in the Leguminosae, e.g., trimeric vicelins of 150–210 kDa as well as hexameric legumin of over 300 kDa). At seed germination, the storage proteins are rapidly hydrolyzed, and the resulting amino acids are transferred to the growing embryo. The aleurone vacuoles are therefore cytolysosomes – compartments for intracellular substance breakdown.

Cell sap spaces often show lytic properties. They contain acidic phosphatase and other lytic enzymes, including proteinases, RNAses, amylase, and glycosidases.

All the functions a vacuole can perform rest on the barrier function of the tonoplast and the specific transport process at the tonoplast. With the aid of isolated vacuoles (*Fig. 2.58a*), the whole spectrum of substance

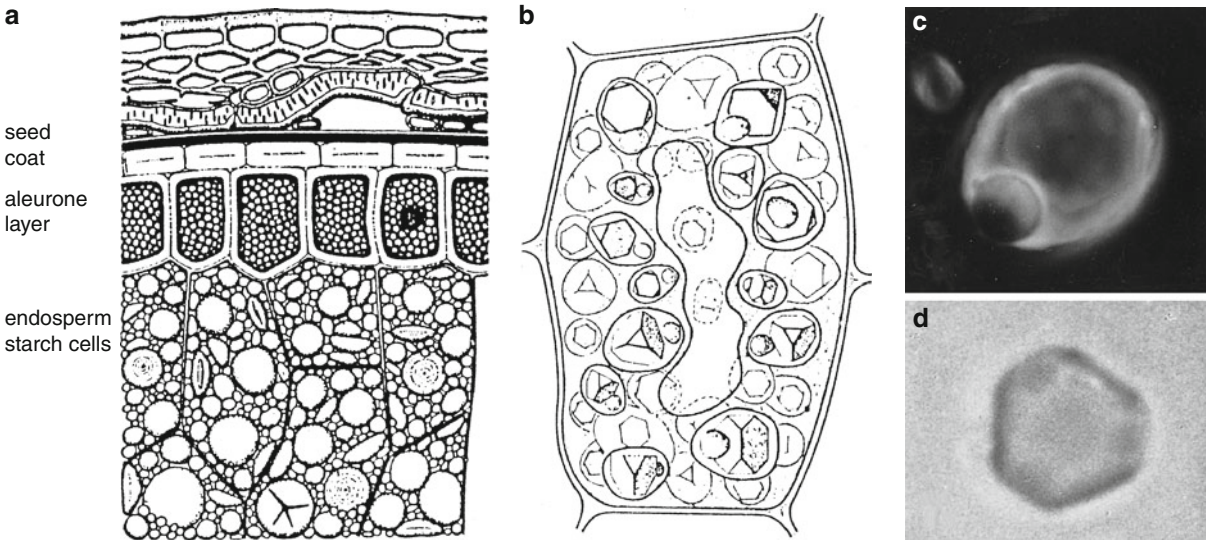

Fig. 2.61

Aleurone grains. (a) Transverse section through the outer layers of a rye grain (×135). (b–d) Endosperm of *Ricinus communis*: (b) cell with central oil vacuoles (castor oil) and numerous aleurone grains, each with tetrahedral protein crystalloids and amorphous globoids (×400); (c, d) isolated aleurone grain or crystalloid (×670) (a After G. Gassner; b after D. von Denffer)

translocation mechanisms in biomembranes can be demonstrated. Antibody marking of intrinsic tonoplast proteins has shown ever more clearly that one and the same cell can have several different sorts of vacuolar compartments.

The **dynamics of cell sap spaces** is considerable. This is the basis of plant organ elongation growth (see Sect. 6.1.1). Mostly, the **central vacuoles** form smaller **prevacuolar compartments** by fusion. During winter, the reverse process can be observed in the cambium cells of woody plants (see Sect. 3.1.2): the central vacuole divides itself into numerous smaller vacuoles that fuse together again during the following spring.

In some cases, a completely different origin has been documented: an organelle-free plasma region is surrounded by ER cisternae that then fuse to form a hollow spherical cisterna.

This is followed by autolysis in the inner space, creating a vacuole. The tonoplast membrane arises from the outer membranes of the ER cisternae.

2.2.7 Cell Walls

The wall of plant and fungal cells, as a shaping **exoskeleton**, provides resistance against the turgor pressure that the protoplasts exert (about 0.5–1 MPa = 5–10 bar) against the cell wall, thus holding the vacuole-bearing cells in mechanical–osmotic equilibrium. The wall itself is a waste product of the living cell that exists in constant interaction with the cytoplasm across the cell membrane. As such, under natural conditions, it forms an integral part of the living cell. From a chemical point of view, it is an association of many different polysaccharides and proteins; from a structural point of view, it is a mixed body composed of an amorphous **ground substance** (**matrix**) and a **structural substance**, a fibrillose scaffold, within it. Most walls of tissue cells are dotted with numerous plasmodesmata – plasma bonds between neighboring cells can be observed at visual limits with a light microscope.

The cell wall belongs to the most characteristic components of plant and fungal cells. They divide by internal cell secretions, forming a cell plate. The existence of an encircling wall around a cell means phagotrophic feeding is not possible. Conversely, in a wall-less cell – a so-called **dermatoblast** – the cytoplasm is predominantly present as a plasma sol (chemical solution). In fact, protoplasm streaming is a frequent occurrence in algal and higher plant cells. Because of the cell wall structure, the cells are not translocated within the plant body. Although unsegmented lactifers or fiber cells are able to squeeze between tissue cells or grow past them, in general, cells do not move around as they do in multicellular animal ontogenesis. Once a plant cell wall has formed, it is very seldom broken down again. A large proportion of the bodies of perennial woody plants are made up of dead tissue (wood, peridermal cork).

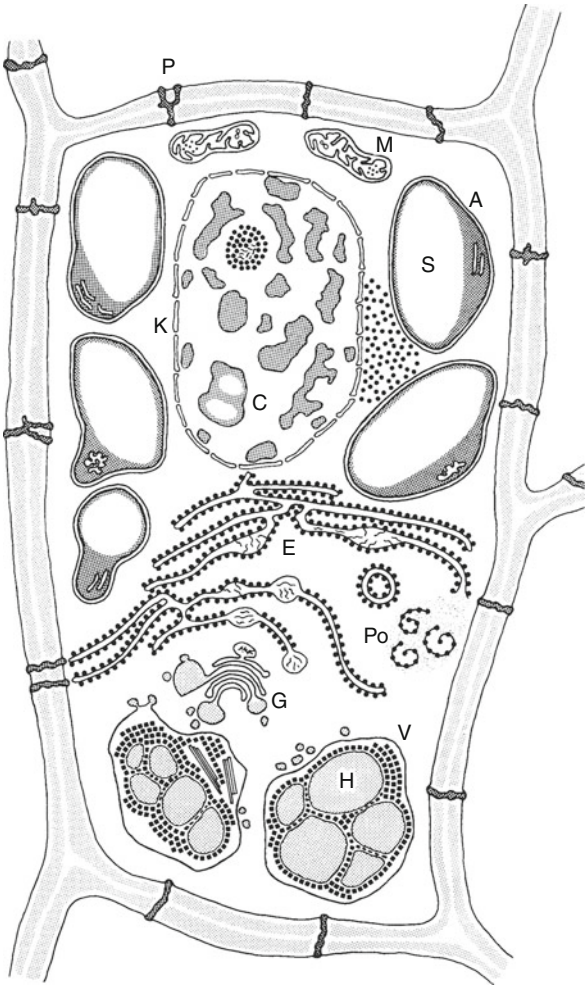

Fig. 2.62

Formation and deposition of storage proteins in barley (*Hordeum vulgare*). Cell nucleus with chromatin and nucleolus; amyloplast with starch; rough endoplasmic reticulum with polyribosomes; dictyosomes with segmented protein vesicles; protein vacuoles with amorphous hordein; and granular globulin. *A* amyloplast, *C* chromatin, *E* rough endoplasmic reticulum, *G* dictyosome, *H* hordein, *K* cell nucleus, *M* mitochondria, *P* plasmodesmata, *Po* polyribosomes, *S* starch, *V* protein vacuole (After D. von Wettstein)

2.2.7.1 Development and Differentiation

The development of the plant cell wall begins at cell division with the formation of the cell plate by the flowing together of Golgi vesicles in phragmoplasts (see Sect. 2.2.3.6). The **cell plate** is composed of a matrix mainly made up of pectins, with a low protein component. This remains permanently as the middle lamella, meaning that any cell wall is basically made up of three layers. The **middle lamella** has no fibril scaffold, which means it is particularly easy to break down. The tissue then breaks down into its individual cells (**maceration**; Latin *maceráre*, chop up, macerate).

Immediately after cell division, each of the daughter cells begins to form wall lamellae that now also contain scaffold fibrils. This creates a plastic or flexible **primary wall**. It accompanies the slow embryonic and rapid postembryonic cell growth, although it is stretched by turgor. Nevertheless, it is still true growth because the primary wall becomes thicker as more wall lamellae are laid down, and its dry weight increases. Studies in cell physiology have shown that the deposition of new wall material, especially of ground substance, causes the plasticity of the primary wall during cell growth and not turgor. However, even the proportion of scaffold fibrils increases until they account for about one quarter of the cell wall's dry weight. The scaffold fibers (in many green algae and higher plants, they are made of cellulose) are flexible but tear resistant. The cell pinches itself into a corset, which although still elastic is no longer plastic or stretchy. Thus, the primary cell wall reaches a stable final condition that is maintained until the cell finally dies. The cell wall in this condition is termed "saccoderm" (Greek *sákkos*, cloak, and *dérma*, skin).

In multicellular organisms, cell differentiation is also expressed in subsequent chemical changes in the **saccoderm** or in the formation of special, additional wall layers. Then one speaks of **secondary cell walls**. Secondary wall layers are deposited or accumulate on the saccoderm. The composition and ultrastructure reflect the function of the individual layers. In land plants, the functions of stabilization and insulation dominate. Mechanical secondary cell walls (see Sect. 2.2.7.4) are found in stabilization tissues, whereas insulating ones are characteristic of dermal or boundary tissues (see Sect. 2.2.7.6).

2.2.7.2 The Primary Cell Wall

Various components of the **cell wall matrix** dominate primary cell walls – pectins, hemicelluloses, and wall proteins (*Fig. 2.63*). The matrix substances are secreted by the Golgi vesicles. The cell wall matrix has low mechanical stability, being an easily swellable isotopic gel with a complex makeup.

Pectins are chemically heterogeneous. Originally, strongly negatively charged acidic polysaccharides (galacturonans and rhamnogalacturonans) were considered

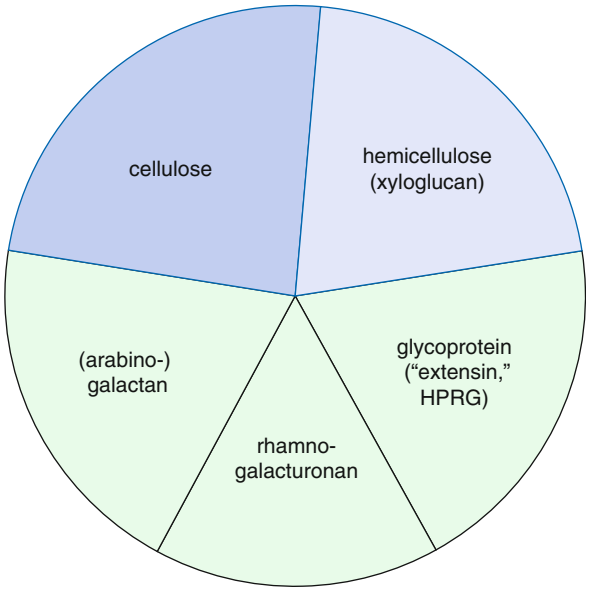

Fig. 2.63
Composition (dry weight) of the primary cell walls from a culture of *Acer pseudoplatanus* (sycamore). The hemicellulose xyloglucan (21.7%) is a companion of the cellulose scaffold fibrils (23.9%). Arabinogalactans and rhamnogalacturonans are the pectins (together 36%). Hydroxyprolin-rich glycoprotein (*HPRG*) (18.9%). The equivalent percentages for cell walls of *Arabidopsis thaliana* leaf are 28%, 14%, 42%, and 14% (After data from P. Albersheim and colleagues)

protopectins, becoming pectins by the esterification of some of the carboxyl groups with methyl alcohol. These days, weak acids are also included among the pectins, as are the strongly hydrophilic and comparatively short chained polysaccharides – arabinans, galactans, and arabinogalactans. Overall, they are characterized by being easily soluble in water and their great swelling potential. In the middle lamella, in particular, the individual molecules are cross-linked with each other by bivalent cations (Ca^{2+}, Mg^{2+}). The pectins dissolve if these ions are removed (e.g., by oxalate or chelators such as ethylenediaminetetraacetic acid). They cause the cell walls to become effective cation exchangers. In some plant organs (especially often, e.g., in seed coats), pectins are mass-produced and are known as gums or slimes (e.g., quinces, gum arabic).

Hemicelluloses are less hydrophilic and generally have larger molecules. To dissolve them, it is necessary to use alkalis. The main representatives of the hemicelluloses include the **glucans** with β(1 → 3) and β(1 → 4) bonds and the **xyloglucans** (in grasses, these are replaced by xylans with attached arabinose or other residues). The xyloglucans are made up of β(1 → 4)-bonded glucose units, most of which carry α(1 → 6)-bonded xylose chains. They sheathe the cellulose fibrils and thus give the cell wall its stability. Their contribution to mechanical secondary cell walls is markedly high.

The enormous heterogeneity of the matrix polysaccharides – especially those components that contributed rather little to the stability of the wall – was puzzling for a long time. It has since been shown that these cell wall substances play a series of important roles. As in animal bodies, where heterosaccharides in the cell surfaces form some of the most important cell detectors and receptors of the cell (e.g., blood group determinants), wall substances play a crucial role in the gamete recognition and the control of the pollen tube growth in the style tissue. The growth of parasitic fungi in plant tissues is inhibited by **phytoalexins**. Phytoalexin synthesis is triggered by elicitors, some of the most potent of which are oligosaccharides released during cell wall breakdown by fungal or host plant enzymes (see Sect. 8.3.4).

The main cell wall proteins are glycoproteins with unusually high levels of hydroxylated proline. Almost all hydroxyproline residues (more than 13 of the amino acid residues) are glycosylated, with tri-L-arabinose or mainly tetra-L-arabinose chains. The proportion of polypeptides in these **hydroxyproline-rich glycoproteins** (**HRGPs**) accounts for only one third of 1% of the 86-kDa molecular mass; the rest is carbohydrate. The protein proportion forms a stiff rod structure of 80-nm length, surrounded by an arabinose sheath. HRGPs have a strong associative tendency. It is assumed that they form a stabilizing network in the cell wall matrix. In a stress situation, e.g., injury or parasite attack, they are produced in greater quantities. However, there are also plants, especially among the monocots, whose cell walls contain only little structural protein. They are often completely lacking in mechanical secondary cell walls.

With regard to the amino acid sequence of the HRGPs, there are surprising parallels to those of collagens – the most important structural proteins of the intercellular matrix in animals and humans. This indicates a shared common ancestry for the gene of these hydroxyproline-rich extracellular structural proteins. Certain algae (e.g., *Chlamydomonas*) have a cell wall that is almost entirely composed of a crystalline layer of HRGP.

The HRGP **extensin** is one of the most widespread structural proteins of primary cell walls. An important subgroup of the HRGPs is the **arabinogalactan proteins**. Less than 10% of the total mass of these proteoglycans is accounted for by proteins. Besides the HRGPs, there are two other classes of cell wall glycoproteins: proline-rich and glycine-rich proteins.

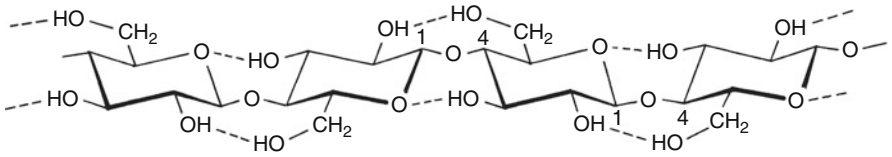

Fig. 2.64

Cellulose, section of the β-1,4-glucan chain: two cellobiose units (equivalent to four glucosyl residues). Hydrogen bonds (*dashes*) are lateral to the main valence chain

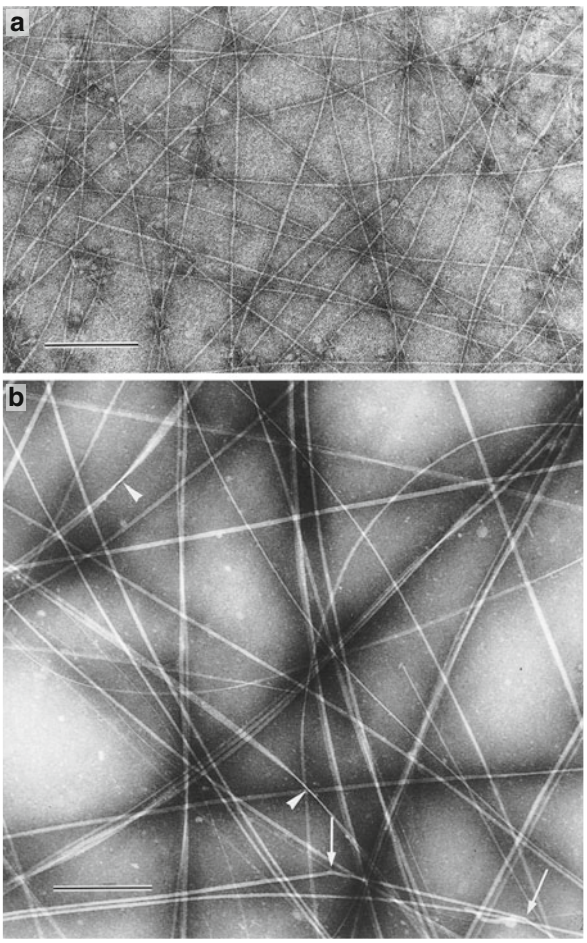

Fig. 2.65

Isolated cellulose fibrils in negative contrast. (a) Elementary fibrils from quince slime. (b) Microfibrils of the siphonal green alga *Valonia*; the differing diameters are partly due to the band shape of these tough scaffold fibrils (*arrowheads*); under a strong bending force, they snap like crystals (*arrows*). *Scale bars* (a) 0.2 μm, (b) 0.4 μm (EM images from W.W. Franke)

The **cell wall scaffold** of all higher plants is made of **cellulose**. Two thousand to over 15,000 β-glucose units form long unbranched straight-chain molecules (in contrast, the α-D-glucan chains of the storage polysaccharides starch and glycogen are spirally twisted and in part branched).

In cellulose, the neighboring glucose units along and around the molecule's axis are each twisted against each other by 180° and held in this position by hydrogen bonds either side of the glycosidic bond (*Fig. 2.64*). The pyranose ring of the individual monomers become aligned in a single plane along the glucan chain as a result of this twisting, thus forming 8-μm-long band-shaped cellulose chain molecules. These molecules have a string associative tendency; they align easily against each other forming hydrogen bonds. They first form elementary fibrils (about 3 nm diameter) and finally form much thicker microfibrils (especially in secondary walls) with diameters of 5–30 nm (*Fig. 2.65*). There are crystal-lattice-like arrangements in these band-shaped scaffold fibrils. In particular, the tougher microfibrils of the secondary wall layers only have a limited flexibility because of their crystalline content, and when bent too sharply, they can snap just like crystal spicules. Functionally speaking, it is important that the scaffold fibrils are very tear resistant. A 1-mm-thick, compact cellulose thread could bear 60 kg (i.e., take a tensile strain of 600 N); this is equivalent to 80% of the tensile strength of steel.

The unusually strong optical anisotropy of cellulose is due to the strict parallel orientation of the cellulose molecules in the scaffold fibrils, and it is expressed in a distinctive birefringence in cellulose-rich wall layers. Additionally, the crystalline fibrils in cellulose cause a pronounced diffraction pattern in X-ray images. These are just two more ways in which the cell wall scaffold differs diametrically from the isotropic amorphous cell wall matrix.

The biosynthesis of cellulose occurs in rosette-shaped, seldom linear, protein complexes in the plasma

membrane (Fig. 2.66). Every **cellulose synthase** complex (terminal complex) forms several cellulose chains that crystallize to form elementary fibrils immediately after their synthesis. Thick microfibrils arise from the concentrated activity of a number of neighboring synthase complexes.

Synthesis and fibril formation are, under natural conditions, tightly coupled together, although it is possible to separate them artificially. Pigments (e.g., Congo red and calcofluor white) that bond particularly strongly with cellulose molecules inhibit crystallization; cellulose synthesis continues but no fibrils are formed.

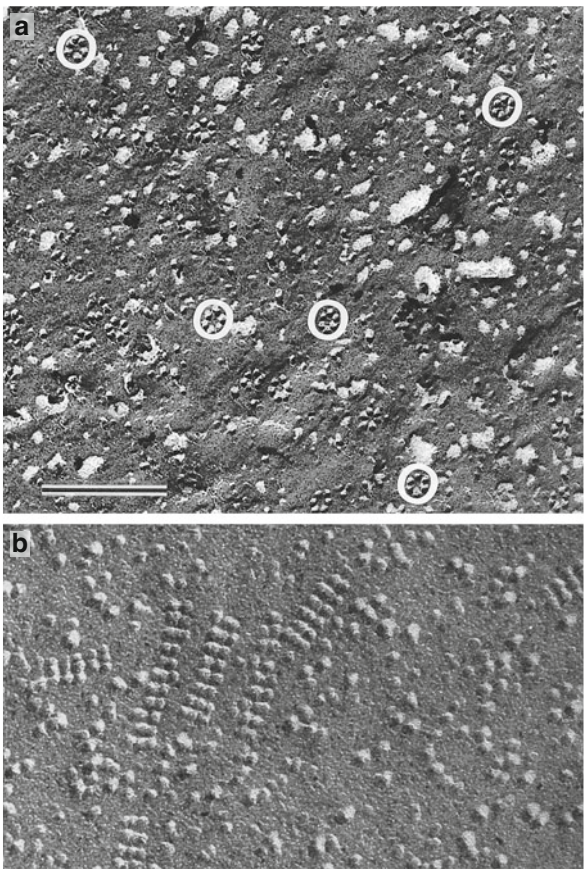

Fig. 2.66
Cellulose synthase complexes. (a) In the cell membrane of the foliose moss *Funaria hygrometrica* (protonema). Of the 20 rosettes shown, five are *circled*. (b) "Linear" complexes in the red alga *Porphyra yezoensis*. *Scale bars* 0.1 μm (Freeze-fracture preparation by and EM images from a U. Rudolph and b I. Tsekos and H.-D. Reiss)

The tunicates, the only animal group that forms cellulose microfibrils (tunicin), have linear cellulose synthase complexes in the outer cell membranes of their epidermis cells.

The C-1 atoms of the individual glucose units all point in the same direction along the molecule axis. The cellulose molecules of native elementary fibrils and microfibrils are in this sense all oriented in the same way (cellulose I) – a consequence of their simultaneous genesis at the synthase complexes. The parallel orientation is not the most efficient position energetically speaking. The frequent precipitation of cellulose from solution in a technological process [e.g., the production of copper rayon from cellulose solutions in ammoniacal copper(II) hydroxide, Schweizer's reagent] produces fibrils whose molecules run antiparallel; this cellulose II is more stable because it is more energy poor than the native cellulose I.

Cellulose is the most common organic macromolecule in the biosphere; over 10^{11} t of cellulose is synthesized annually. The economic significance of cellulose and its numerous derivatives is enormous, especially in the textile industry, but principally in the biofuel industry. Pure cellulose is harvested from the seed hairs of cotton and from various procedures involving wood. However, cellulose has no nutritional value for humans, and cellulose-rich diets are very rich in fiber. Many herbivores, especially the ruminants, have special adaptations to digest cellulose. Endosymbiotic bacteria and ciliates that produce cellulase play an important role in this case.

Chitin, a linear polymer of *N*-acetylglucosamine, occurs principally in the animal kingdom (arthropods), but also in many fungi and some algae as an extracellular scaffolding substance. Despite the other monomers, chitin fibrils have a construction similar to that of cellulose. Their stability is even greater than that of cellulose because of the intensive interlocking of neighboring chain molecules.

In the multinucleate giant cells of the siphonal marine algae, cellulose as a structural substance is substituted by xylans or mannans. These polysaccharides are able to form crystalline aggregates, but the fibril formation is not as pronounced as with cellulose or chitin. Cellulose is the scaffold substance for all larger, photosynthetically active land plants. It can be instantly formed from photosynthetic products. Nitrogen-containing scaffold substances (chitin, protein) are favored in heterotrophic organisms – nitrogen is never in short supply.

The predominantly crystalline cellulose of chitin fibrils does not allow the uptake of water. In contrast, the amorphous, hydrophilic matrix substances can swell or dry out according to the availability of water. In the absence of water, they shrink to thick, horny masses; with water, they form jellylike masses whose dry weight often does not even equal 3% of the fresh weight

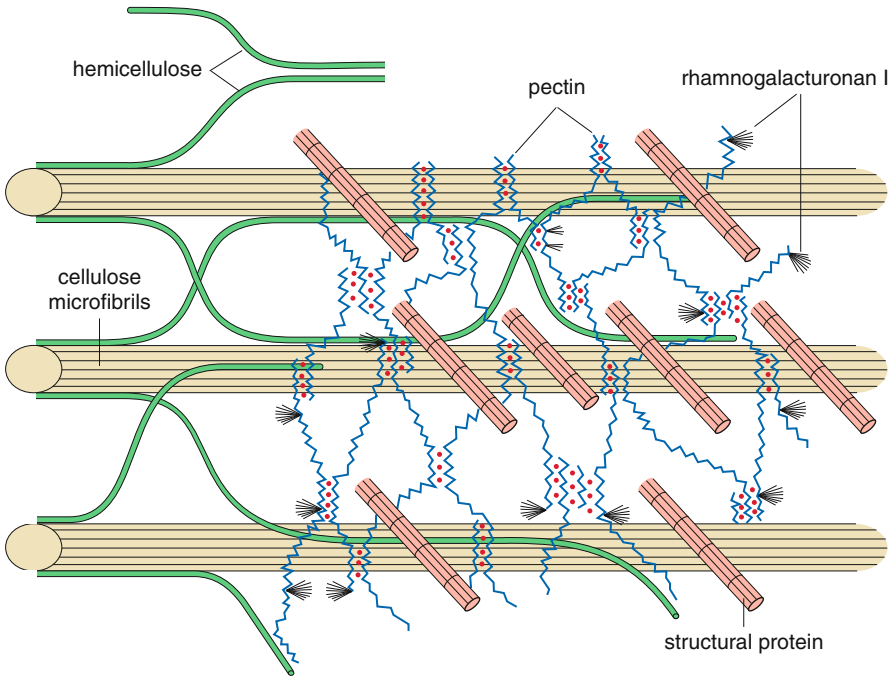

Fig. 2.67

Simplified diagram of the molecular structure of the primary cell wall. Of the many cell wall components, only the cellulose microfibrils (tightly bonded to the cell wall by hydrogen bridges) and the cross-linked xyloglucan chains (hemicellulose, *green*), pectins (cross-linked by Ca^{2+} ions, *red*), and cell wall proteins are illustrated (Modified from C. Brett and K. Waldron, from L. Taiz and E. Zeiger)

(this property is deliberately used in the production of nutrient media, e.g., agar, alginate, and jellies). **Hygroscopic movements** of cell walls or tissues (see Sect. 7.4) are entirely due, on the one hand, to the unchangeability of the scaffold fibril lengths and, on the other hand, to the swelling ability of the matrix substances. This is immediately obvious when the scaffold and the matrix do not permeate throughout the cell together as in primary walls but rather are separated, like in the well-known spore haptera of horsetails (Fig. 10.169h, j). Their hygroscopic behavior is due to an inner layer of cellulose being layered onto a swellable arabinoglucan layer.

Molecular structural models of the primary cell walls (*Fig. 2.67*) refer to the cellulose fibrils having a sheath of particularly resistant xylans or xyloglucans. These cross-link the fibril scaffold. Pectins form a second, thickening mesh in the links of the network. Although the average mesh size (porosity) of a native primary wall is about 5–10 nm (maximum value 20 nm; globular proteins up to about 50 kDa can permeate), the pectin-extracted wall will let particles of up to 40 nm diameter pass through.

During **surface growth** of the primary cell wall, the successively deposited wall lamellae gradually become more stretched. At the same time, new wall lamellae are constantly being produced by the cell. In the process, every wall lamella within the cell wall is pushed further to the outside, becoming ever thinner, and the mesh of the wall scaffold becomes ever looser. The growing cell determines the direction of the scaffold fibrils in the forming wall lamellae. Every incipient scaffold fiber is trapped between the plasma membrane and the preexisting wall lamella. This means that the cellulose synthase complexes have to push themselves backward in the fluid plasma membrane because of their synthesizing activity. This shifting activity is almost certainly directed (in the manner of a crash barrier) from cortical microtubules located in the membrane (*Fig. 2.71*). Many growing cells whose scaffold fibers in successive primary wall lamellae are twisted by a fixed angle undergo a full twist in 1 day – an impressive expression of a circadian rhythm (see Sect. 6.7.2.3).

Cell wall plasticity is controlled by growth factors (see Sect. 6.6.1.4) and is important for their surface growth.

It is based on the reduction of the transverse meshing of the scaffold fibers, ensuring they slide past each other and can also avoid each other.

Primary cell walls contain various enzymes that can cause this type of looseness. Glucanases can break down matrix glucans in the presence of cellulases. Hydrogen-bridge bonds between cellulose fibrils and xyloglucans are temporarily broken by **expansin.** Other enzymes can cause lengthening of the xyloglucan chains by adding on more monomers.

Various components of the cell wall are directly associated with the cytoskeleton via integral cell membrane proteins. These bridge proteins are characterized by the sequence Arg-Gly-Asn (RGD). If the bond between the cytoskeleton and the exoskeleton is upset by this sequence as a result of excess peptide supply, the cells are no longer capable of dividing regularly nor are they able to assort themselves correctly in a tissue.

The final form of plant and fungal cells depends on whether the primary cell wall grows isometrically or anisometrically. Cells that grow strongly in one direction have **apical or terminal growth**. In this case, the secretion of matrix substances – performed by exocytotic Golgi vesicles – is restricted to the cell tip, where the cytoplasm is especially rich in actin filaments. Cells with apical growth can push their way through spatially fixed structures, e.g., ground particles in root hairs and fungal hyphae, or between neighboring cells of fibers, lactifers, and pollen tubes (**intrusive growth**).

2.2.7.3 Plasmodesmata and Pit Fields

Plasmodesmata are plasma links between neighboring cells that go through the separating cell walls. They connect the single cells in tissues to a symplastic continuum. Plasmodesmata often occur in groups called **primary pit fields**, so called because they can become closing membranes for the pit fields (see Sect. 2.2.7.5). The cell walls are often thinner in this region (*Fig. 2.68c*). Every plasmodesma in the cell wall is surrounded by a callose coat. Callose is easily identifiable using fluorescence microscopy. This means that plasmodesmata, despite their small diameters of 30–60 nm, can be easily located using a light microscope (*Fig. 2.68a*). Under the electron microscope, they appear as simple or branched tubes (*Figs. 2.68d–g*, *2.70*) surrounded by cell membrane. The plasma membranes of the connected cells intergrade with each other. Every plasmodesma is threaded through by a central strand, the **desmotubule** (*Figs. 2.68e*, *2.69*). This is a local modification of the ER. Unlike its misleading name suggests, it is not a tubular structure but rather is a compact strand of structural proteins that makes contact with ER cisternae in adjacent cells. The hollow, cylindrical space between the desmotubule and the cell membrane is part of the cytoplasm compartment. According to its size, it should also allow the passage of large protein molecules, and in special instances this is indeed the case; e.g., plant viruses spread through tissues via the plasmodesmata. Normally, its permeability is restricted to molecules of less than 1-kDa molecular mass (diameter 2 nm) because junction proteins are clamped between the globular proteins of the cell membrane and the appropriate proteins in the desmotubule, splitting the plasma cylinder into a number of narrow microcanals. These can be enlarged by special **motor proteins** that mediate specific, directional transport (see Sect. 6.4.4.1, Fig. 6.30).

Many plasmodesmata are already present during cell division as recessions in the cell plate (**primary plasmodesmata**). Plasmodesmata are also continually produced later, so their numbers per unit area remain constant even in growing walls (**secondary plasmodesmata**) even though the wall area expands during postembryonic cell enlargement to over 100 times the original area. During grafting or parasite attack (e.g., by *Cuscuta*; Figs. 4.38, 4.39a, 10.150d), plasmodesmata can form between cells from individuals of different races or even different species. Secondary plasmodesmata – frequently branched – are formed as outlined in *Fig. 2.70*.

In a 100-µm^2 wall area, there can be 5–50 plasmodesmata in the parenchyma. When neighboring cells cooperate closely with each other, such as companion cells and sieve tubes in the phloem (see Sect. 3.2.4.1) or mesophyll and bundle-sheath cells in C_4 plants (see Sect. 5.5.8), much higher numbers of plasmodesmata are reached; in meristems, these can number over 1,200 per 100 µm^2. Conversely, in physiologically isolated cells, e.g., stomatal guard cells (see Sect. 3.2.2.1), they are particularly rare.

Should mass flows between cells be possible, then the secondary plasmodesmata are especially amplified. The most well known example is given by the **sieve pores** in the sieve plates of the phloem sieve tubes (see Sect. 3.2.4.1). The size of these wall perforations can, in extreme cases, reach 15 µm, although values between 0.5 and 3 µm are typical. Conversely, plasmodesmata can be sealed or even completely broken down (in the presence of ubiquitin). If a cell in a tissue dies, then its plasmodesmata are rapidly pushed and closed by thickening of the callose coat, allowing the adjacent cell to survive unaffected. Even original plasmodesmata located between living cells or groups of cells can disappear when these symplastic domains are isolated from their surroundings as part of

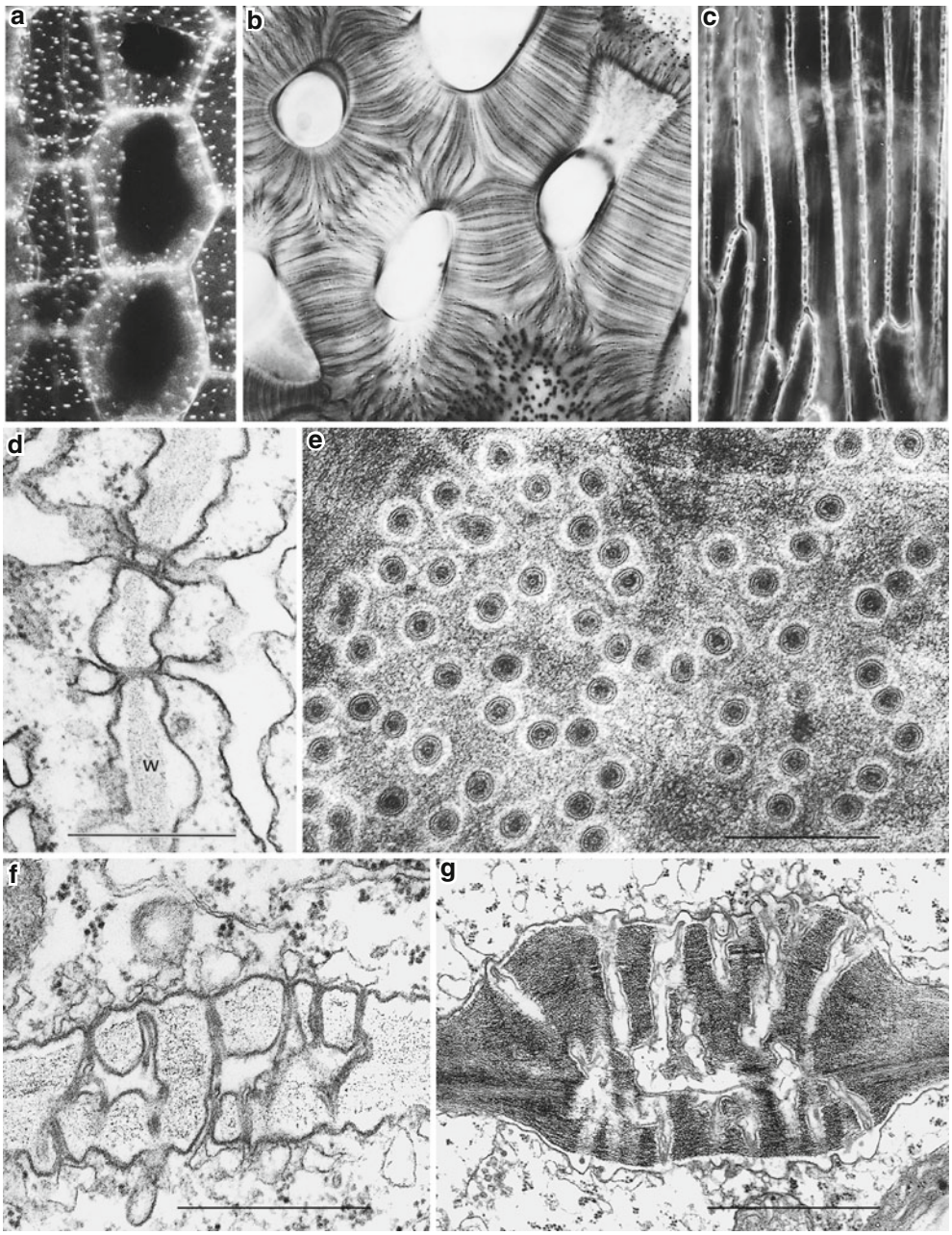

Fig. 2.68

Plasmodesmata and primary pit fields under the LM and the EM. (a) Aniline blue fluorescence of the callose in the shoot parenchyma of the pumpkin *Cucurbita pepo* (×220). (b) Iodine–silver impregnation contrasted in thickened cell walls in the endosperm of *Royena villosa* (×770). (c) Dark-field image of cell walls in the pod septum of honesty *Lunaria rediviva*; the apparent perforations are thinner sites equivalent to primary pit fields (×300). (d) Plasmodesmata with rough endoplasmic reticulum contact in the wall between callus cells of *Vicia faba* (*scale bar* 0.5 μm). (e) Transversely sectioned plasmodesmata of a primary pit field in *Metasequoia glyptostroboides*; each plasmodesma is separated from the light callose coat in the cell wall by a trilaminar cell membrane with a central desmotubule. (f, g) Modifications of primary plasmodesmata between Strasburger cells in the needles of *Metasequoia glyptostroboides*, early developmental stage and final stage (*scale bars* 0.2 μm). *W* wall (a LM image from I. Dorr; b LM image from I. Dorr and B. von Cleve; d–g EM images from R. Kollmann and C. Glockmann)

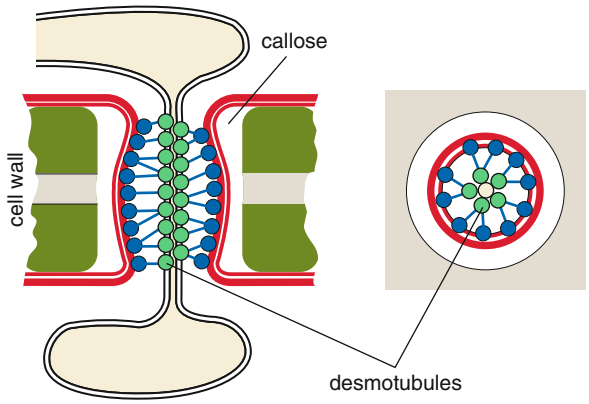

Fig. 2.69

Ultrastructure model of a plasmodesma: *left* longitudinal section, *right* transverse section. *Red* cell membrane (After W.J. Lukas)

a morphological process. Contrary to past opinion, plasmodesmata are not static entities but are highly dynamic structures whose frequency and permeability can be rapidly adjusted to local conditions and demands.

Animal tissues do not contain plasmodesmata. They do have gap junctions which physiologically couple neighboring cells and also special regions of plasma membranes with numerous canals (connexons), each made of six protein molecules (connexin). Plasmodesmata and connexons are analogous structures because, although they are constructed differently, they perform the same functions – exchange of ions and signaling molecules between cells.

2.2.7.4 Secondary Walls of Fiber and Wood Cells

The weight of aquatic plants is compensated for by water buoyancy. Plants that reach into the air must be able to bear their own weight (an exception is climbing plants; Table 4.1). In large land plants, this function is performed by specially developed **strengthening tissues** (see Sect. 3.2.3). Two cell types are to be found in these tissues: fiber cells where there is tensile load and woody cells (e.g., stone cells, tracheids, tracheary elements) with stiff walls when it is necessary to resist an external force or pressure.

The massive secondary thickening layers of the walls of **fiber cells** and some plant hairs (e.g., cotton) are mainly made of densely packed cellulose microfibrils. Their dry weight fraction can reach up to 90% in these walls.

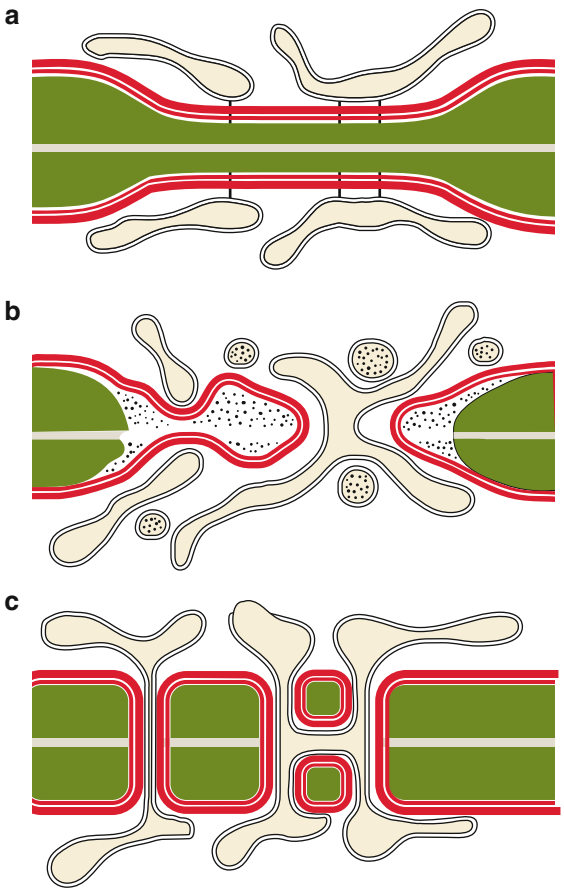

Fig. 2.70

Genesis of secondary plasmodesmata. (a) Endoplasmic reticulum elements of pairs of neighboring cells have approached the cell membrane (*red*); the cell wall (*green*) is broken down at this site. (b) The endoplasmic reticulum elements of both cells fuse; Golgi vesicles deliver new wall material. (c) The newly completed cell wall is interspersed with, in part, branched secondary plasmodesmata ("median nodules" around the middle lamella) (After R. Kollmann and C. Glockmann)

Fiber cells (and some thick-walled hair cells) reflect the typical dimensions and structural properties of the scaffold microfibrils to a high degree: they do not tear even under strong stress but remain flexible. This is why plant fibers are so important economically. Because the secondary wall layers are only laid down in the inner surface after surface area growth of the primary cell wall has been completed, the cell lumen (space) is constricted by the extent of wall thickening. In the end, the space available

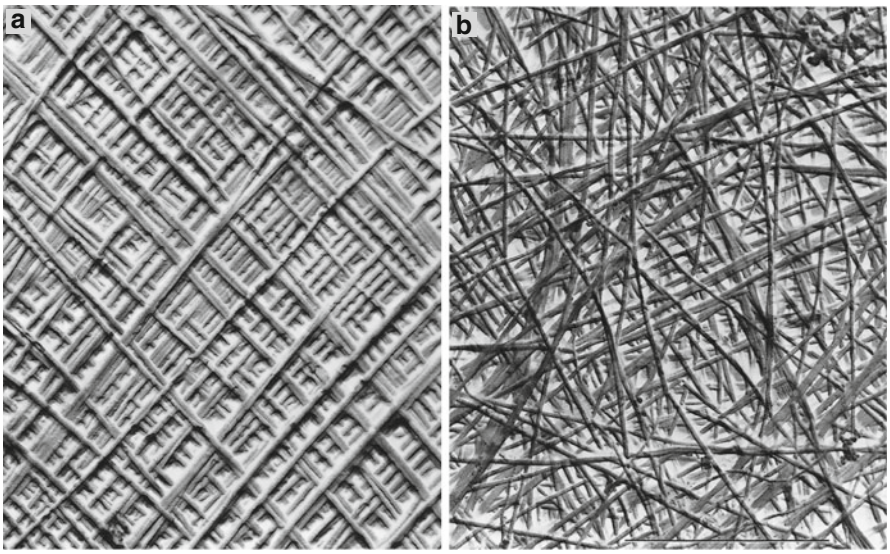

Fig. 2.71
Parallel texture and scattered texture of cellulose microfibrils. The cell wall of the alga *Oocystis solitaria* is composed of many superimposed lamellae. (a) Normally, the scaffold fibrils in each lamella would run parallel to each other and rotate by 90° between lamellae (*cross-hatched texture*). (b) Colchicine, under whose influence the cortical microtubules disband, causes a scattered texture. *Scale bar* 1 μm (EM images from D.G. Robinson)

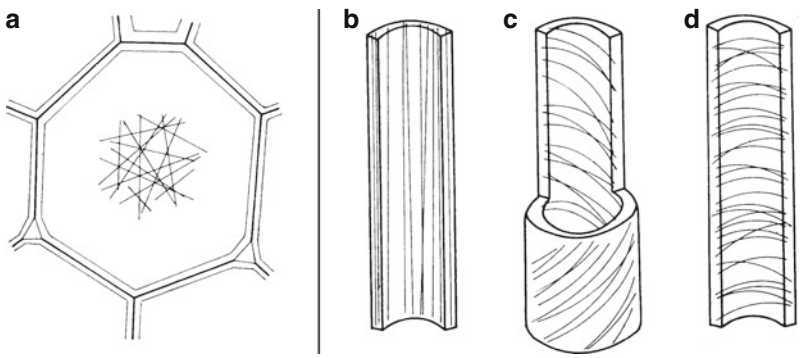

Fig. 2.72
Arrangement of the cellulose microfibrils in cell walls. (a) Scattered texture is typical of the isodiametric cells of the saccoderm. Secondary wall lamellae have a parallel texture. (b) Fibrous texture. (c) Spiral texture – the most common form. (d) Tubular texture

for the living protoplast can be reduced to less than 5% of its original volume; then the cell dies. Only the wall coat has functional significance.

Cellulose microfibrils are oriented parallel to the cell membrane. Within this area there are, however, various possible arrangements (**textures**; Latin *textúra*, tissues, weave; *Figs. 2.71*, *2.72*). Whereas the primary wall usually has a scattered (sheet) texture that is frequently directional, the lamellae of secondary wall layers are characterized by having **parallel texture**. Elongated cells such as fiber cells can be differentiated into fibrous, spiral, and tubular textures, depending on the direction of the microfibrils with respect to the longitudinal axis of the cell. Tubular and fibrous textures are borderline cases of spiral

textures. The direction of texture is that of the greatest tensile load.

The walls of fiber cells often have bold spiral textures which, unlike cells with the more uncommon strictly longitudinal or fibrous texture, are able to withstand sudden tensile events. The twist direction of the spiral textures differs. The secondary wall fibrils of hemp and jute fibers are right-handed spirals (Z-spirals), whereas those of flax and nettles are left-handed spirals (S-spirals). (right-handed spirals mean that the spiral tracks follow the central part of the Z, from the eye of the viewer; left-handed spirals follow the central part of the S). Plant hairs with thickened walls can change the spiral direction a number of times. In the centimeter-long cotton hairs, this can happen up to 150 times.

Not surprisingly, tubular textures never occur in fiber cells. The name originates from the lactifers found in many plants (see Sect. 3.2.5.1) and refers to the typical walls found therein. The lactifers are internally pressurized, and although the liquid pressure is isotropic, the transverse wall tension is greater than the longitudinal wall tension.

The apposition of secondary wall layers occurs spasmodically. Lamellae are formed that often grow incrementally on a daily basis and form packets of lamellae called **secondary wall layers**. The general construction and the common names for the layers are given in *Fig. 2.73*. After the saccoderm, there comes a comparatively thin secondary wall layer, the S1 layer (a transition layer) with flat spiral texture. This is immediately followed (to the inside) by the thick S2 layer, composed of up to 50 wall lamellae. This layer is crucial from a functional point of view. The densely packed scaffold microfibrils have a spiral or fibrous texture. Toward the cell lumen, there is a final thin S3 layer (tertiary wall) with yet another different texture. This can be coated in a structurally and chemically different isotropically homogeneous warty layer.

In firm, pressure-resistant cell walls, the scaffold fibrils are packed or encrusted in a firm material. These **encrusted cell walls** primarily contain **lignin**, as well as mineral substances (such as silicates in Poaceae and calcium carbonate in Dasycladaceae). Lignin encrustation means the cell wall is woody (Latin *lignum*, wood) or has become **lignified**. The lignins – there are three different chemical forms in monocot, broad-leaved, and coniferous woods – arise in the lignifying cell wall by polymerization of phenol bodies (monolignols; see Sect. 5.16.2, Figs. 5.129–5.131), which for their part are then exocytosed by the Golgi vesicles as soluble glucosides. The giant molecules of lignin grow in all directions, proliferating and rampaging through the microfibril scaffold of the cell wall. Lignin macromolecules aggregate

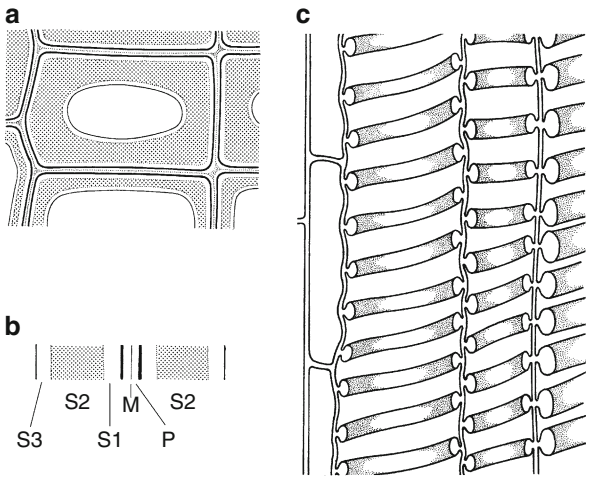

Fig. 2.73
Secondary wall thickenings in tracheid walls of a conifer. (a) Transverse section (×800). (b) Cell wall layers. *M* middle lamella, *P* primary wall (saccoderm), *S1* transition lamella, *S2* actual secondary wall, composed of many lamellae, *S3* tertiary wall. (c) Spiral tracheids in pumpkin with characteristic thickened bars belonging to the S2 layer (see also Fig. 3.24e); *left* parenchyma cells (a, b After I.W. Bailey)

secondarily to form huge units and expand beyond the middle lamella, meaning that the lignin mass of a trunk is possibly a single gigantic polymer molecule whose mass should be measured in tons. During lignification, the original cell wall matrix is replaced or suppressed by the compact lignin polymers. Lignified cell walls typically consist of two thirds cellulose and resistant hemicelluloses (mostly xylans; Greek *xýlon*, wood) and one third lignin.

The cellulose fibrils become so densely packed in lignin that they are no longer able to move against each other, and they completely lose their already restricted swelling ability. Whereas the cellulose in primary walls can be loosened by the addition of a concentrated solution of, e.g., zinc chloride so that it is able to take up iodine and thus stains deep violet, this reaction fails in lignified cell walls. The superb strengthening properties of such cell walls and of lignified tissues, particularly of wood itself, is due to the reciprocal penetration of tear-resistant, flexible scaffold fibrils with the stiff, dense filler lignin.

Pressure loads are also present in the water transport vessels, in the woody part (xylem) of vascular bundles, and in the wood of perennial stems or roots. The water transport xylem elements (tracheids and tracheary elements;

see Sect. 3.2.4.2) arise from living cells but in their functional condition consist of dead, lignified cell wall tubes. Thanks to their lignification, the xylem strands and wood are often the most important weight-bearing structures of the vegetative bodies of land plants.

2.2.7.5 Pits

Lignification not only stiffens the cell walls but also makes them less permeable. Whereas the nonwoody primary walls can be penetrated by particles of up to 5-nm diameter, even the water permeability of woody cell walls is reduced. This is also significant for the water transport systems in the roots, stems, and leaves: lignification means that the lateral movement of water in and out is impeded. **Pit canals** (pores or wall channels of light-microscope dimensions) are formed wherever water conduction (or general substance exchange) is necessary. *Figure 2.74* shows typical pit structures in secondarily thickened cell walls. The pores of neighboring cells correspond to each other – they meet at primary pit fields. The still present primary wall and middle lamella function as **closing membranes** for the pits.

Bordered pits are characteristic of water transport vessels. The secondary wall layers around the pores are withdrawn by the closing membrane, forming a funnel-shaped "pit." Conifer tracheids are typified by having distinctly large, circular pit fields; water rising up the bole flows through them. The closing membranes are thickened toward the middle to a torus or cushion supported by loose radial threads of cellulose. Water is able to flow between the supporting spokes from one tracheid into another. Bordered pits act as one-way valves in air embolism events, by pushing against the torus on the low-pressure side, effectively closing it (*Fig. 2.74c*; Choat et al. 2008).

2.2.7.6 Isolating Secondary Walls

One of the most important criteria for plant life (and active life in general) is the constant availability of water (see Sects. 5.3, 12.5). Most land plants have special "facilities" to reduce their drying out in air. Those cells with lipophilic secondary wall layers that are located at the surface (epidermis cells) or near the surface (cork cells) of the vegetative body are particularly important. In contrast to the mechanically strengthening secondary walls that always contain a lot of cellulose, the sealing secondary wall layers are composed of impermeable, hydrophobic material and typically do not contain cellulose. Impermeability to water is due to the **accrustation** of lipophilic masses on a particular saccoderm, which serves as a base for the accrustation and provides the necessary mechanical stability. Accrustations are made of **cutin** (on epidermises; Latin *cutis*, skin, surface) and the chemically related **suberin** (on cork cells; Latin *suber*, cork; see Sect. 5.16.3). Cutin and suberin form a polymer matrix in which particularly hydrophobic wax components are stored.

The **corky cell wall** is made up of a cellulose-free suberin layer on the inside of the saccoderm (*Fig. 2.75*). On the cell lumen side, it is usually covered in a third, thin layer that contains cellulose (tertiary wall). The functionally important role is played by the **suberin layer**, as an accrusted, secondary wall layer. It is practically impermeable, even to water. This is due to the wax, which is stored in 3-nm-thick lamellae that run parallel to the surface of the suberin layer (*Fig. 2.76a*). The rod-shaped wax molecules are perpendicularly oriented to the plane of the lamella. Once the wax has been removed (mostly esters of fatty acids with wax alcohols), what remains is the insoluble, amorphous, isotropic polymer matrix – the suberin. This matrix is a three-dimensional meshed condensate of long-chain fatty acids, fatty alcohols, and related compounds. It is only weakly hydrophobic and is water-permeable. It functions as a stable support for the delicate wax films, which for their part block the passage of hydrophilic substances. The lamellar construction of the secondary wall ensures that even with defects in individual layers, the whole functions as a very effective barrier.

The molecular building blocks of suberin and wax lamellae are secreted (granulocrine; eccrine; Greek *krínein*, deposit, secrete) from the cork cells not via Golgi vesicles but by diffusion. Their formation site is in the smooth ER. The formation of the suberin layer can happen quite fast, e.g., closing injured sites in just a few hours.

The **cuticle** has, in principle, a construction similar to that of the suberin layer of cork cells (see Sect. 3.2.2.1). It is also a cellulose-free, lipophilic wall layer with surface-parallel wax films in a cutin polymer matrix. The whole complex is also deposited on the primary cell wall (*Fig. 2.76b*). Differently from the suberin layer, the accrustation is on the outer surface of the saccoderm. The molecular building blocks are secreted through the primary wall to the outside by the epidermis cells, a process which involves the small basic lipid transfer proteins in the wall matrix. Thus, the epidermis cells share a continuous accrusted wall layer – the cuticle.

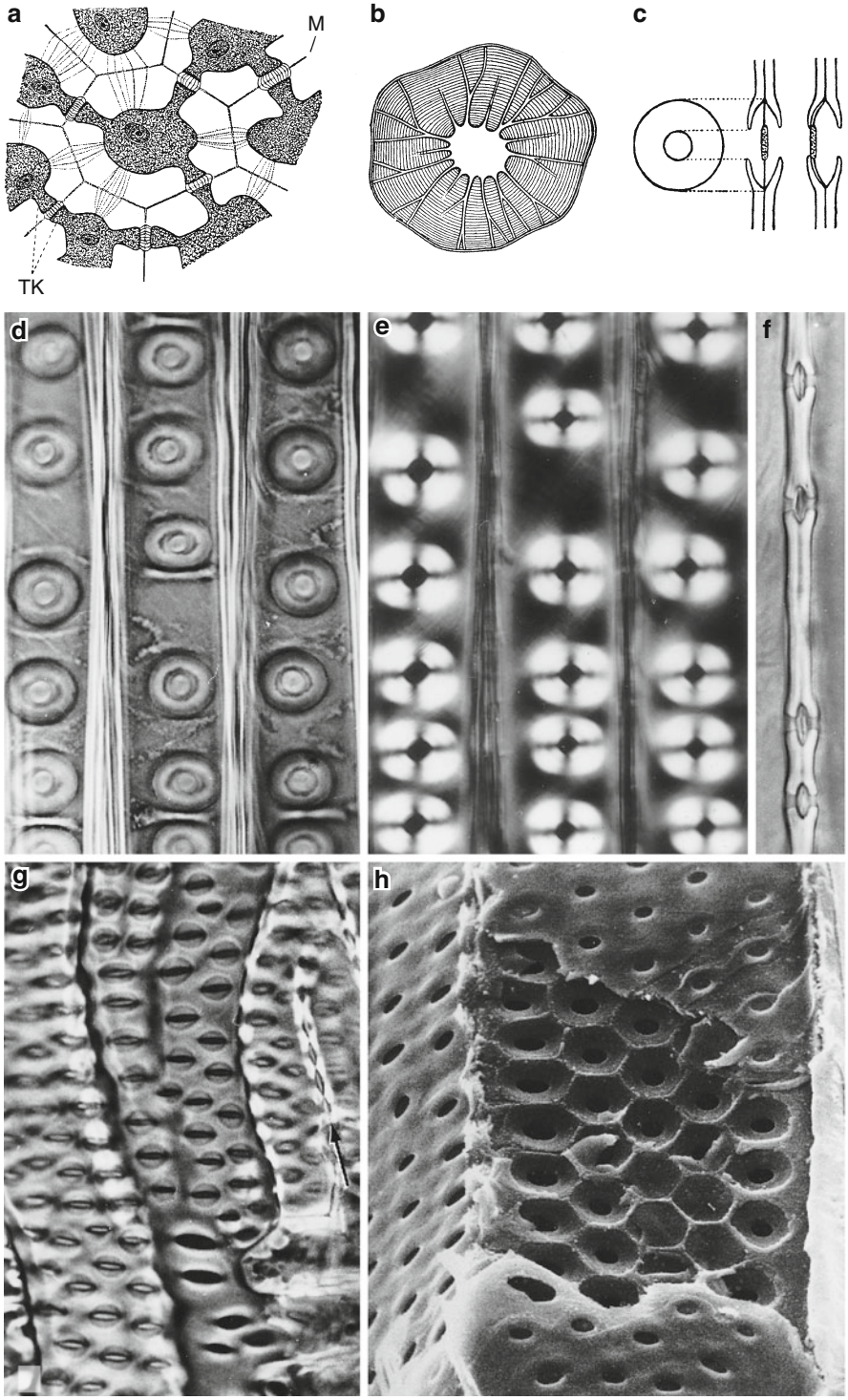

Fig. 2.74

Pits. (a) Section taken from the "stone endosperm" of the Ivory palm *Phytelephas*; the strongly thickened cell walls serve here as a depot for storage polysaccharides; the cells are connected by plasmodesmata and also between pit channels (×230). (b) Stone cells (sclereids) from a walnut shell with branched pit channels which do not apparently penetrate all

The cuticular waxes have longer hydrocarbon chains than the cork waxes and are accordingly more hydrophobic (the number of carbon atoms ranges between 25 and 33, compared with 18–28 in cork waxes).

Particularly, in plants from arid habitats, there are wax crystals on the cuticle surface (**epicuticular waxes**; see Fig. 3.11), making the cuticle unwettable. Often, cutin masses are deposited in the outer lamellae of the primary epidermis cell walls below the cuticle itself. In these **cuticular layers**, the cutin and the accompanying waxes occur as encrustations. The poor mixing ability of these hydrophobic wall substances with the hydrophilic components of the primary wall is expressed by the relatively poor ultrastructure. The wax films are often broken up and no longer run parallel to the surface, and the additional transpiration protection is only moderate, even in thick cuticle layers. Similar phenomena are not found in the cork cells; in the multilayered cork tissue, better sealing can be achieved simply by increasing the number of corky layers, whereas the cuticle is restricted to the air-bordering epidermis surface and is therefore basically single-layered.

The body surface of arthropods also has a cuticle. Whereas the massively developed inner layers of the insect cuticle (endocuticle and exocuticle) form as chitin-containing exoskeleton that provides the mechanical stability, the outer encrusted epicuticle has a high concentration of wax in it and thus provides excellent transpiration protection. This epicuticle shares many chemical and ultrastructural properties with the plant cuticle – an impressive example of convergent evolution in animals and plants.

Even the generally microscopically small spores and pollen grains have encrusted cell walls (**sporoderms**; Figs. 10.198, 10.200). Encrustations include the particularly resistant **sporopollenin**. Its function is not to keep water back (this would be illusory with the high surface area to volume ratio, and these cells can survive complete desiccation anyway), it rather functions as a protective layer, e.g., able to absorb damaging UV radiation. Sporoderms are entirely different from the cutin walls of epidermises and the suberin layers of cork cells, not only functionally, but also with respect to their chemistry, ultrastructure, and development. They are important in classification and for the reconstruction of vegetation history (pollen analysis).

Callose is a special kind of sealing material. It is a glucan with $1 \rightarrow 3$ binding of the monomers, which have spiral molecules and always occur in a very compact form without addition of other substances. Callose is used to close plasmodesmata and sieve pores (see Sect. 2.2.7.3); callose can be rapidly synthesized in large quantities at the plasma membrane and can be just as quickly broken down again. Frequently, callose assumes the role of a protective bandage, at a cellular level.

2.2.8 Mitochondria

Some general structural mitochondrial data are summarized in *Fig. 2.77*:

- There is a **double envelope** composed of two different membranes that have a nonplasmatic compartment between them, the **intermembrane space**. The inner mitochondrial membrane forms characteristic folds (**cristae**) that are commonly narrow at their base, but in the mitochondria themselves, they enlarge somewhat, becoming slightly inflated (*Fig. 2.78*). In some cases, the cristae form a spatial network.
- There are elementary particles on the inside of the inner mitochondrial membrane that correspond to the mitochondrial **ATP synthase complexes** visible under the electron microscope (*Fig. 2.79*). They are composed of a stalk-like foot (F_0 complex) that reinforces the function of the mitochondrial membrane as a proton channel and the head-shaped F_1 complex, which is the actual ATP synthase complex. During ATP synthesis, the whole complex rotates around its long axis oriented perpendicular to the membrane surface.

secondary walls but run obliquely across the section plane (×670). (c–f) Bordered pits in conifers: (c) diagrammatic representation – *left* overview, *center* longitudinal section, *right* longitudinal section showing valve function under unilateral pressure; (d, e) bordered pit of Scotch pine *Pinus sylvestris* overview, in phase contrast and under the polarization microscope (the cellulose fibrils ring the black pore; the concentric structure, thus gives a dark cross – see also *Fig. 2.89b*) (×330); (f) bordered pits of mountain pine *Pinus mugo*, longitudinal section – pit formation by removal of the secondary walls, pores, and the closing membrane with a torus are recognizable (×600). (g, h) Bordered pit in deciduous woods: (g) with slit pores (''cat's eyes'') in vessel walls of the pedunculate oak *Quercus robur*, *right* also wall transverse section (*arrow*; ×530); (h) pit vessel in willow wood (*Salix*) (×1,000). *M* middle lamella, *TK* pit channels (a After W. Halbsguth; b after W. Rothert and J. Reinke; f LM image from H. Falk; h SEM image from A. Resch)

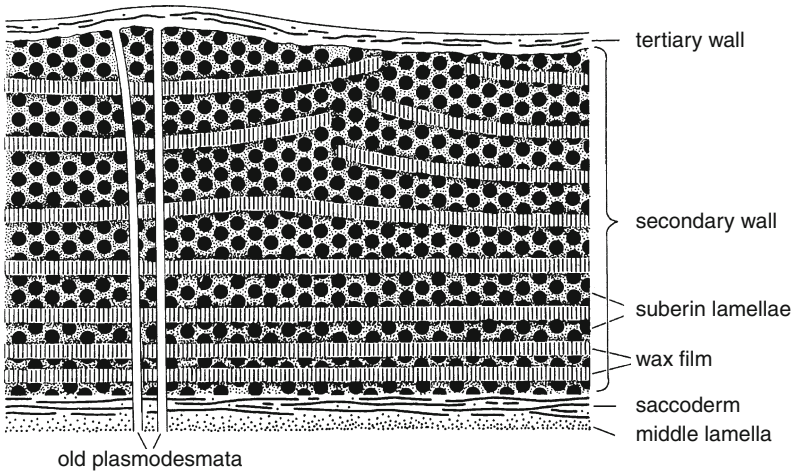

Fig. 2.75
Ultrastructure model of a corky cell wall. The lipophilic suberin layer does not contain any cellulose. There are scaffold fibers in the tertiary wall

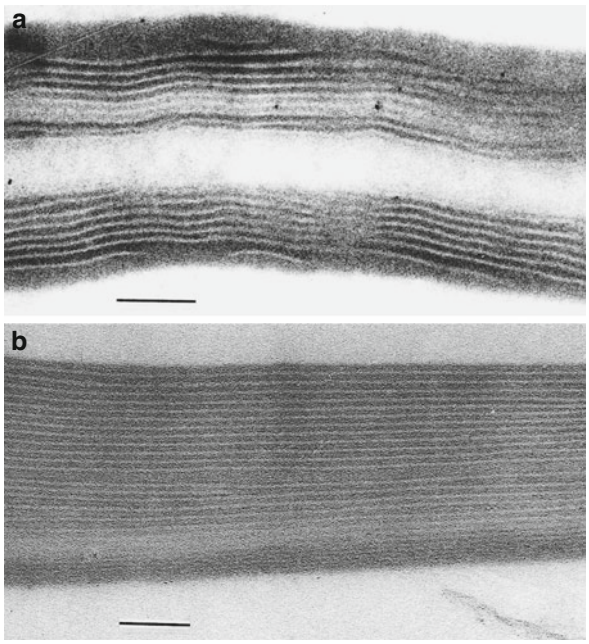

Fig. 2.76
Lamella structure with accrusted cell wall layers (transverse section); wax films not contrasted, polymer matrix (suberin, cutin) *dark*. (a) Cell wall of wound cork of the potato; walls of two adjacent cork cells with lamellated suberin layers. (b) Separated cuticle of *Agave americana*. *Scale bars* 0.1 µm (EM images from a H. Falk and b J. Wattendorff)

- There is a matrix with **70S ribosomes** and circular **mtDNA**. Mostly, there are a number of, often many, DNA rings in the organelle. They are concentrated in distinct, relatively loose regions of the organelle matrix, which in analogy with the structural organization of bacteria are called nucleoids. There are no histones or nucleosomes.
- Occasionally, dense matrix granula occur in which, among other things, calcium and magnesium ions are stored.

2.2.8.1 Shape Dynamics and Reproduction

In thin-layer sections (*Fig. 2.78*) and after isolation (*Box 2.1*, *Fig. B2.1*), mitochondria commonly appear as spherical or elliptic bodies of about 1-µm diameter. In postembryonic living cells, typically filamentous, elongated, and sometimes even branched mitochondria are observed (*Fig. 2.80*).

Mitochondria have the ability to rapidly alter their shape. In yeasts and also in some algae, the numerous mitochondria of a cell fuse together during certain developmental stages or under certain external conditions to form a single, reticulate giant mitochondrion that later splits into small individual mitochondria. Fusion and multiple divisions of mitochondria are also not uncommon in higher plants.

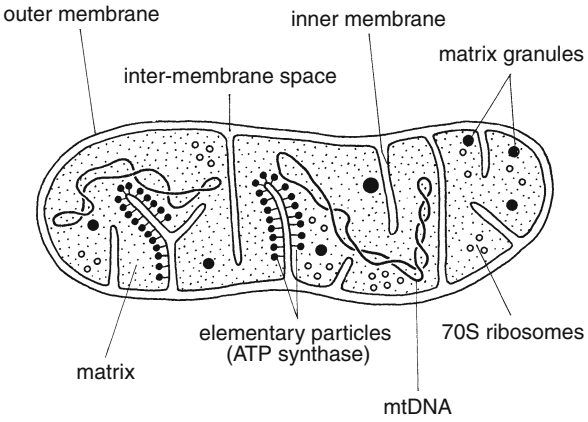

Fig. 2.77

A mitochondrion. Inner and outer membranes differ not only in their structure and enzyme complement but also in their lipid composition (cardiolipin/cholesterol, see also *Fig. 2.99*). The inner membrane folds to form cristae, on the mitoplasmic (matrix) side of which ATP synthase complexes are located. *mtDNA* mitochondrial DNA (After H. Ziegler)

Mitochondria can only arise from mitochondria. Their **reproduction** occurs mainly in the meristems. Reproduction is based on the binary fission of the organelle body after a septum has been formed in the intermembrane space (*Fig. 2.81*). The sheer number of mtDNA molecules in the mitochondrion ensures that no daughter mitochondrion will be formed without having genetic information in it. Rapid cell replication often results in an incomplete enzyme complement of a mitochondrion; the resulting **promitochondria** are not respiratorily active.

In yeasts, there are also DNA-free mitochondria that are nevertheless capable of reproducing. Yeasts are facultative anaerobes; they are able to survive without oxygen and do without respiration or mitochondria – a rare exception among the eukaryotes. For this reason, mutational changes in the mtDNA that result in respiration-defective yeast mitochondria are not automatically lethal. The so-called *petite colonie* mutants of brewer's yeast *Saccharomyces cerevisiae* occur spontaneously with a frequency of 1–2%; mutagens such as acriflavine and ethidium bromide can raise the mutation rate to nearly 100%. The "petites" have a respiratory disorder; under aerobic conditions, they grow significantly more slowly than wild-type yeasts; their colonies on solid glucose agar remain smaller, thus their name. The mtDNAs of the "petites" are either garbled (damaged;

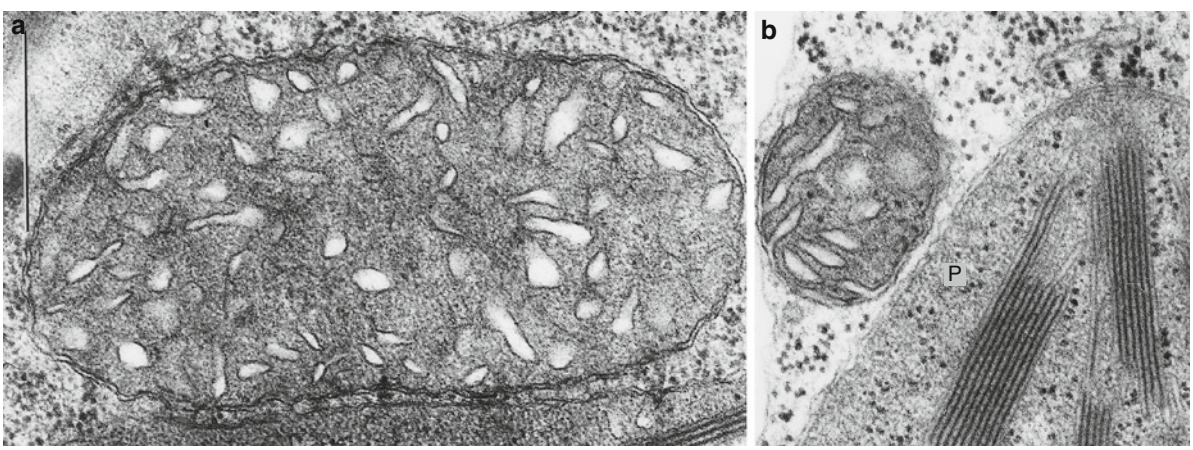

Fig. 2.78

Mitochondria in the EM. (a) In the leaf cell of spinach, numerous sections of cristae can be seen whose nonplasmatic inside is in contact with the intermembrane space of the double membrane sheath, such communications are not visible here because they are outside the plane of the section. In (b), they are clearly identifiable. The mitochondrial ribosomes are – like the plastid ribosomes (*P*) in the chloroplast – significantly smaller than the cytoplasmic ribosomes. *Scale bar* 0.5 µm (EM images from H. Falk)

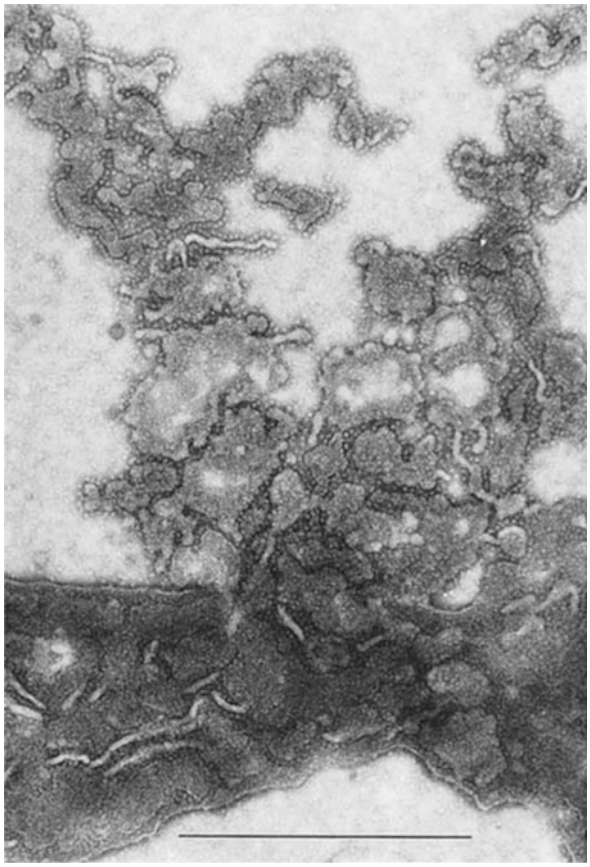

Fig. 2.79
ATP synthase complexes in the cristae membranes are clearly visible as light "elementary" or F1 particles in this negative-contrast preparation of isolated, burst mitochondria from potato tissue. They are connected to the membranes by delicate stalklets (F0, not visible here). The molecular construction is similar to that of the chloroplast ATP synthases (see Fig. 5.59). *Scale bar* 0.5 μm (EM image from H. Falk)

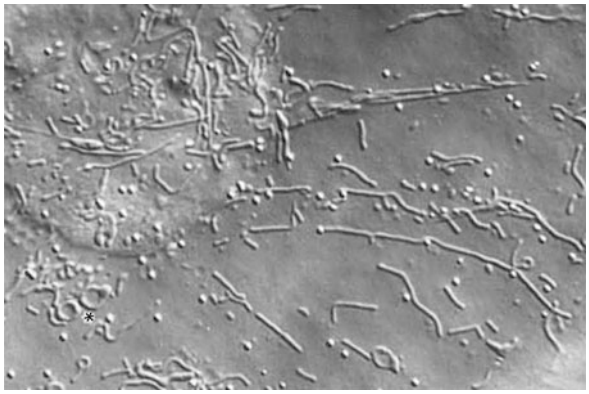

Fig. 2.80
Mitochondria are able to rapidly change their form in living cells. Mostly, they occur as threads of sausage-shaped structures as shown here in the inner (*upper*) bulb epidermis of *Allium cepa*. As well as numerous "spaghetti mitochondria," there are also short mitochondria and spherical oleosomes and several leucoplasts with starch-like invaginations (example marked with an asterisk). *Top left*, an unclear cell nucleus (×670) (Interference contrast image from W. Url)

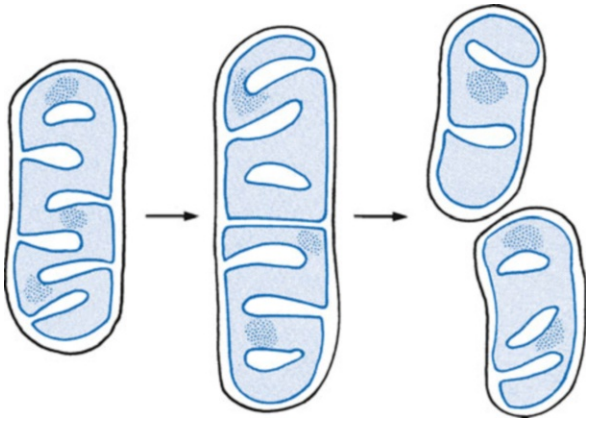

Fig. 2.81
Division of a mitochondrion; nucleoids are *dotted*

ρ^- mutants) or are entirely absent (ρ^0), and certain proteins and components of the multienzyme complexes cannot be formed anymore.

mtDNAs in fungi and plants have very different molecular masses or total lengths (20 to over 800 μm) even though they have about the same gene complement. These differences are determined by the occurrence of noncoding partial sequences (see Sect. 6.2.1.3). In numerous plants, the mitochondria contain variously sized mtDNAs because of intramolecular recombination processes, although incomplete copies may also occur (Fig. 6.7).

2.2.8.2 Membranes and Compartmentalization in Mitochondria

Mitochondria are principally the organelles of **cell respiration**; their most important function is the provision of chemical energy in the form of ATP (see Sect. 5.9.3).

The ATP is derived from an endogenic reaction between ADP and phosphate. The site of this **oxidative phosphorylation** is the ATP

synthase complex on the inner mitochondrial membrane. The energy required is acquired from an **electron transport** sequence in the inner mitochondrial membrane, from energy-rich respiration substrates to oxygen (**respiratory chain**; see Sect. 5.9.3.3). A proton gradient is created at the inner mitochondrial membrane in association with the electron transport, and the pH value sinks in the intermembrane space. At the same time, a membrane potential develops across the membrane, negative inside compared with outside. Proton gradients and membrane potentials are discharged by ATP formation in the rotating ATP synthase complexes. That the energy-rich intermediate stages of metabolic chains not only occur as energy-rich molecules but also as ion gradients and membrane potentials forms the central tenet of P. Mitchell's **chemiosmotic theory**, which also holds true for phosphorylation in chloroplasts (see Sects. 5.4.9, 5.9.3.3). This theory gives prominence to the significance of compartments in cellular energetics.

The electrons for the electron transport in the respiratory chain come from the oxidation of acids in the **citric acid (Krebs) cycle**. Almost all the enzymes used in this cycle are found in the mitochondrial matrix.

Apart from their involvement in cell respiration, plant cell mitochondria are also involved in other metabolic activities, in particular, photorespiration (see Sect. 5.5.6) and programmed cell death (apoptosis; see Sect. 6.3.2).

Outer and inner mitochondrial membranes differ enormously with regard to their integral proteins. Even their lipid complement is different. Whereas the outer membrane contains the commonly occurring (in membranes of eukaryotes) cholesterol, the inner membrane has none and instead has a significant content of cardiolipin, a phospholipid that otherwise only occurs in bacterial membranes (*Fig. 2.99*). An explanation for these relationships is given by the endosymbiont theory (see Sect. 2.4).

The permeability of the outer mitochondrial membrane is high. It contains tubular complexes of integral membrane proteins (**porins**) that allow hydrophilic particles of up to 1 kDa to pass through (as a comparison, ATP has a molecular mass of 0.5 kDa). In contrast, the inner sheath membrane is even impermeable to protons – otherwise, the energizing of the ATP synthases would not be possible. To reconcile the low permeability with the demands of the metabolic exchanges, the inner membrane is generously equipped with numerous specific translocators. These ensure, e.g., the exchange of ATP and ADP (**adenylate translocator**), and also of phosphate and organic acids.

The **protein import** from the cytoplasm into the mitochondrion is a special form of membrane transport. More than 95% of the more than 200 mitochondrial proteins, and even some RNAs (e.g., tRNAs), cannot be synthesized in the mitochondrion itself. Nuclear-coded mitochondrial proteins are generally synthesized in the cytoplasm as unfolded precursor molecules with a **transit peptide** at their amino end. This serves as a recognition sequence, allows the posttranslational attachment of the precursor to an integral **translocator complex** (TOM and TIM) on the mitochondrial envelope (one TOM complex in the outer membrane and two different TIM complexes, TIM22 and TIM23, in the inner membrane) and also the subsequent passing through of the polypeptide. The transit itself occurs at sites where the inner and outer sheath membranes temporarily touch. When the protein reaches its functional position, the transit peptide is split apart and, thus, the protein assumes its final conformation and activity (see Sect. 6.3.1.4).

2.2.9 Plastids

Plastids occur in various forms in one and the same plant. This can be seen macroscopically because of various **pigmentations**: the proplastids in the meristems and the leucoplasts in the ground and storage tissues are colorless; the photosynthetically active, chlorophyll-rich chloroplasts are green, and the gerontoplasts of the autumn leaves and the chromoplasts in flowers and carpels are yellow to red because of carotenoids. All forms of plastids are interchangeable, except the gerontoplasts, which are the end stage of an irreversible development. Like mitochondria, plastids also have a double **plastid envelope**, separating them from the cytoplasm. Similar to the mitochondria, the outer membrane is much more permeable than the inner membrane, which is accordingly equipped with lots of specific translocators. The inner plastid envelope is also the main site of lipid synthesis in plant cells.

Plastids reproduce by **division**. Like in bacteria, the process involves a binary fission event in the organelle with the help of a central contractile ring zone. It contains the **protein FtsZ**, which has the same function as in bacteria and is homologous with tubulin in terms of structure and sequence (see Sect. 2.3.1).

Plastids have genetic continuity, thanks to their own genetic information that is in the form of **plastid DNA** (ptDNA; *Figs. 2.85*, 6.5; see Sect. 6.2.1.2). Like in mitochondria, the coding capacity of the organelles' DNA is not sufficient for the coding of all the plastid-specific proteins. The genes for more than 90% of these proteins are located in the cell nucleus, and the precursor polypeptides that are synthesized on free polysomes in the cytoplasm have to be translocated through the plastid envelope to their target. This is a process similar to that seen in mitochondria, assisted by N-terminus transit

peptides on the preprotein and the corresponding translocator complexes on outer (TOC) and inner (TIC) plastid membranes (Fig. 6.18). Despite having the same function, the proteins of these complexes are only vaguely similar to those of the mitochondria. Chloroplasts have extra facilities for ensuring the correct assembly sites of thylakoid proteins. Whereas a single translocation system operates in the plastid envelope, four different systems have been identified for the correct deposition and assembly of thylakoid proteins.

The theory that plastids possess their own genetic information was developed in the first decade of the twentieth century by E. Bauer and C. Correns on the basis of observed strictly maternal inheritance of streaky green defects in *Antirrhinum* and *Mirabilis* (see Sect. 9.1.2.5). Syngamy in these plants does not result in the male gamete bringing plastids to the zygote. (In other plants, e.g., in *Pelargonium* and *Oenothera*, the plastids are inherited biparentally.) The existence of ptDNA was first verified in the 1960s, and ptDNA was isolated as circular DNA double helix before being characterized. In 1986, two Japanese research groups succeeded in completely sequencing the ptDNA from tobacco (Fig. 6.5) and from the liverwort *Marchantia*; since then, the ptDNAs of many plants have been sequenced (see Sect. 6.2.1.2).

2.2.9.1 Form and Ultrastructure of Chloroplasts

Chloroplasts are the characteristic organelles for all photoautotrophic eukaryotes. Owing to photorespiration during photosynthesis (see Sect. 5.4), the radiant energy of the sun is converted into chemical energy, laying the basis for all organotrophic (heterotrophic) organisms. At the same time, carbon, hydrogen, and phosphorous are assimilated, nitrate and sulfate are reduced, and oxygen is liberated from water. Almost all the oxygen (a requirement for the aerobic harvesting of energy from organic food and the formation of an ozone layer in the upper atmosphere) in Earth's atmosphere comes from photosynthesis.

The internal membranes of typical chloroplasts (*Fig. 2.82*), the **thylakoids**, contain various carotenoids and protein-bound chlorophylls. In contrast to the mitochondrial cristae, the thylakoids are not in direct contact with the inner membrane envelope of the organelle. Photosynthetic photorespiration takes place in the thylakoids. The thylakoids are frequently stacked upon each other (**grana**) in limited areas and in the intermediate regions, the **stroma**; the thylakoids occur singly (*Fig. 2.83*). The molecular structure of the thylakoid membrane reflects its function. The rich protein complement (*Figs. 2.84*, 5.52) and the precise, asymmetrical arrangement or orientation of the protein complexes are a morphological expression of the photorespiration process. ATP formation occurs on plastid ATP synthase complexes (see Fig. 5.59), which resemble the mitochondrial ATP synthases in their structure and which are localized on stroma thylakoids.

The **stroma matrix** is the plasma phase of the organelle. It has been shown that there is a plastoskeleton of FtsZ, which functions to stabilize the chloroplast shape (*Fig. 2.82c*). The stroma matrix contains not only enzymes for the "dark" reaction of photosynthesis (see Sect. 5.5) but also starch grains and other storage structures such as plastoglobuli as lipid stores and under some circumstances protein crystals (e.g., like those of the iron-storage protein phytoferritin). In the stroma matrix, there are several to many nucleoids, diffuse regions with aggregations of ptDNA molecules bearing **plastomes** (*Fig. 2.85*, see Sect. 6.2.1.2) and the 70S ribosomes.

This structural plan of the chloroplasts is often varied to a greater or lesser extent, especially in algae. This is the case in the first instance for the outer shape of the organelle. Whereas the chloroplasts of deciduous higher plants are lens-shaped with diameters of between 4 and 10 µm, and occur as several to many typical chlorophyll grains in a cell, some green algae have particularly large, oddly shaped chloroplasts, often only one per cell (*Fig. 2.86*). The chloroplasts of many algae and the hornworts contain sharply delimited thickenings in the stroma matrix, which are often surrounded by starch grains and in which thylakoids occur either singly or not at all. These matrix regions are called **pyrenoids** (Greek *pyren*, nucleus). They are characterized by having a particularly high concentrations of the key enzyme involved in CO_2 fixation – ribulose 1,5-bisphosphate carboxylase/oxygenase. This enzyme, a complex of eight large and eight small subunits, accounts for significant proportions of the mass of the stroma matrix; in green leaf tissue, often over 60% of all soluble proteins (for its function, see Sect. 5.5.1).

Not all chloroplasts have the grana/stroma arrangement. In the granaless, **homogenic chloroplasts**, thylakoids are not stacked (this is generally true for the plastids of red algae), or there are thylakoid stacks (each with two to three thylakoids) scattered throughout the whole plastid (*Fig. 2.87*).

The plastids of red algae are distinguished by having not only individual thylakoids but also special forms of the protein pigment complexes that function as light collectors for photosynthesis. Whereas these complexes would normally only be visible in thylakoids (*Fig. 2.84*) in planar view (in freeze-fracture preparations), the light collector complexes made of phycobiliproteins

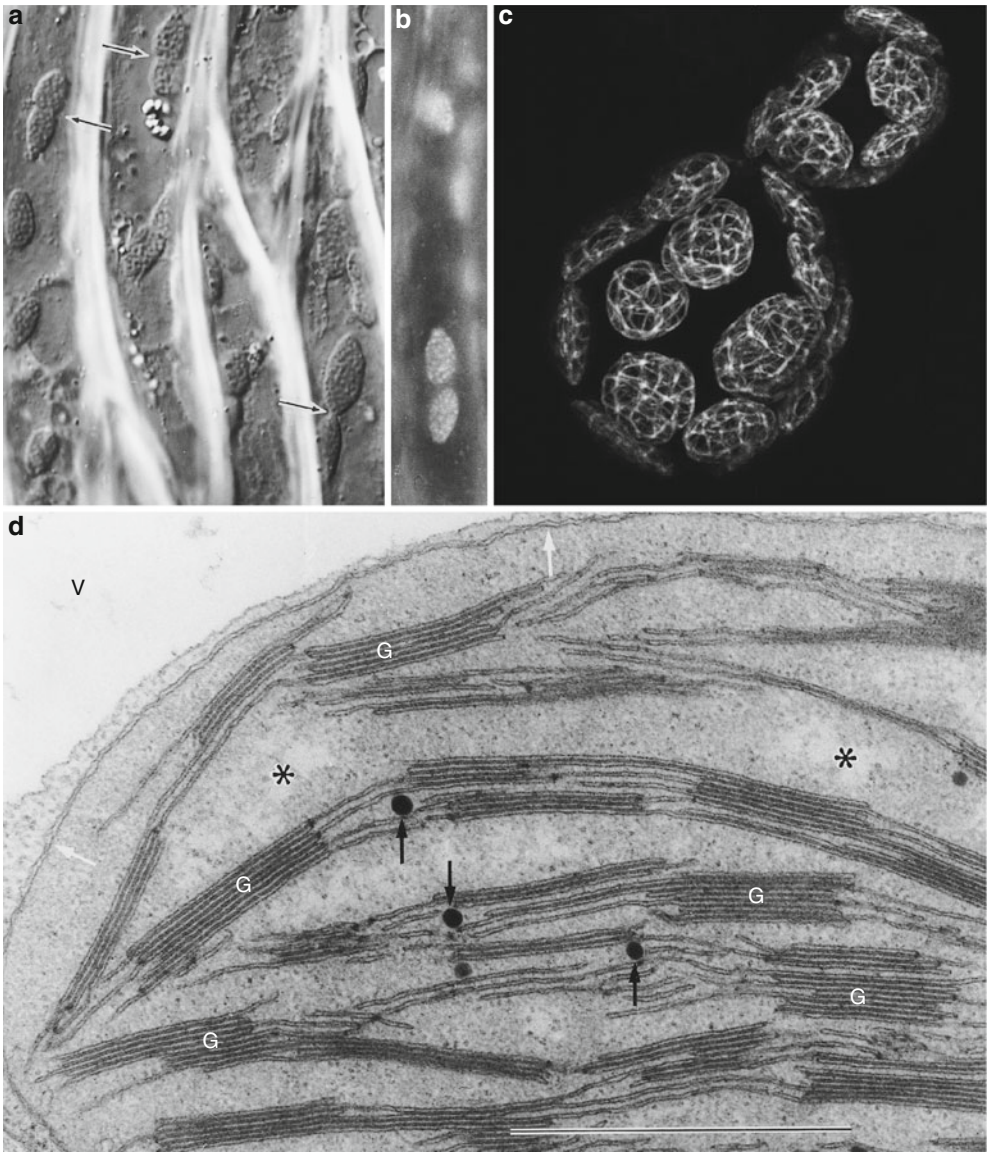

Fig. 2.82

Chloroplasts under the LM and the EM (a, b) Granular chloroplasts in living leaf cells of the moss *Fontinalis antipyretica* (×1,230): (a) chloroplast division by median pinching (*arrows*); (b) chlorophyll fluorescence of the grana. (c) Plastoskeleton in the chloroplasts of the moss *Physcomitrella patens* (regenerative protoplast, living, FtsZ1 fluorescing by GFP; see Sect. 2.1.1) (×2,050). (d) Granular chloroplast from the foliage leaf of a green bean under the EM. The numerous thylakoids are visible as flat, double membranes: in the grana (some identified with *G*), they are densely stacked; between the grana, they are nonstacked stroma thylakoids. *Black arrows* plastoglobuli; scattered areas of stroma matrix (*asterisks*) contain plastid DNA (ptDNA) (''nucleoids''); *white arrows* double plastid envelope. *Scale bar* 1 μm, *G* grana, *V* vacuole (c Confocal laser scanning microscope image from J. Kiessling and R. Reski; d EM image from H. Falk)

(**phycobilisomes**) extend out of the thylakoid plane (*Figs. 2.88*, 5.48b). Corresponding light collector complexes are also found in the photosynthetic membranes of the prokaryotic cyanobacteria. The chloroplasts of not only the red algae (the phycobilin content colors them red–purple: rhodoplasts) but also many other algal groups contain so many accessory pigments (i.e., additional to chlorophyll) that they no longer appear green. This is true for the phaeoplasts of the brown algae and the yellow

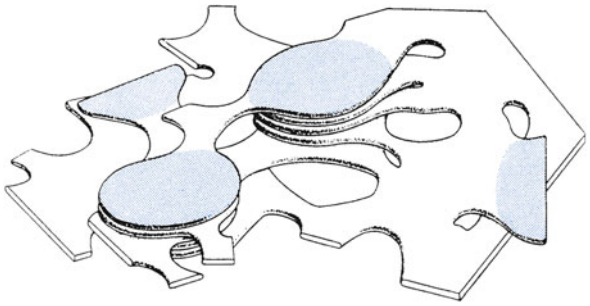

Fig. 2.83
Grana and stroma thylakoids are not special compartments but rather provide a spatial continuum with numerous overlapping membranes. *Blue* grana (After W. Wehrmeyer)

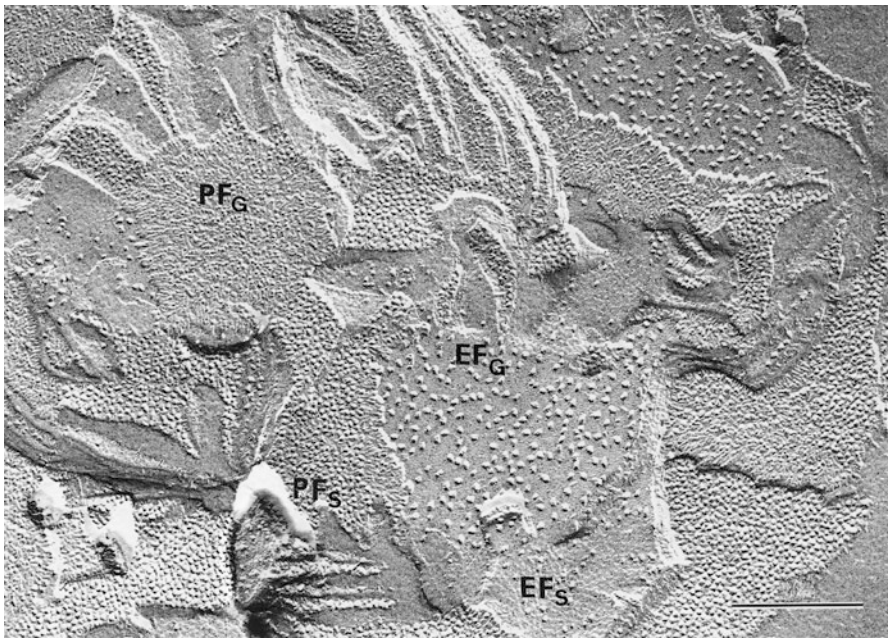

Fig. 2.84
Thylakoid membranes bear protein complexes that are involved in the light reactions of photosynthesis (see Fig. 5.52). Freeze-fracture preparations (pea chloroplast) clearly show these complexes as membrane particles. *EF* exoplasmic face, *PF* protoplasmic face, *G* grana region, *S* stroma region; the functional differences between these thylakoid regions are also apparent in their particle patterns. *Scale bar* 0.3 μm (Preparation and EM image from L.A. Staehelin)

plastids of the dinoflagellates and many chrysophytes. A commonly applied umbrella term for all pigmented plastids is **chromatophore**.

The deeply pigmented **stigma** (colored by special carotenoids) of many flagellates corresponds to a dense collection of pigmented plastoglobuli (Figs. 10.83a, 10.114a). This aggregation of lipid droplets is either localized in chloroplasts or lies outside the plastids in the cytoplasm; presumably, the latter are also plastids, but over the course of evolution have been heavily modified.

2.2.9.2 Other Plastid Types, Starches

The structural and functional variability of the plastids in higher plants far exceeds that of the mitochondria. Any

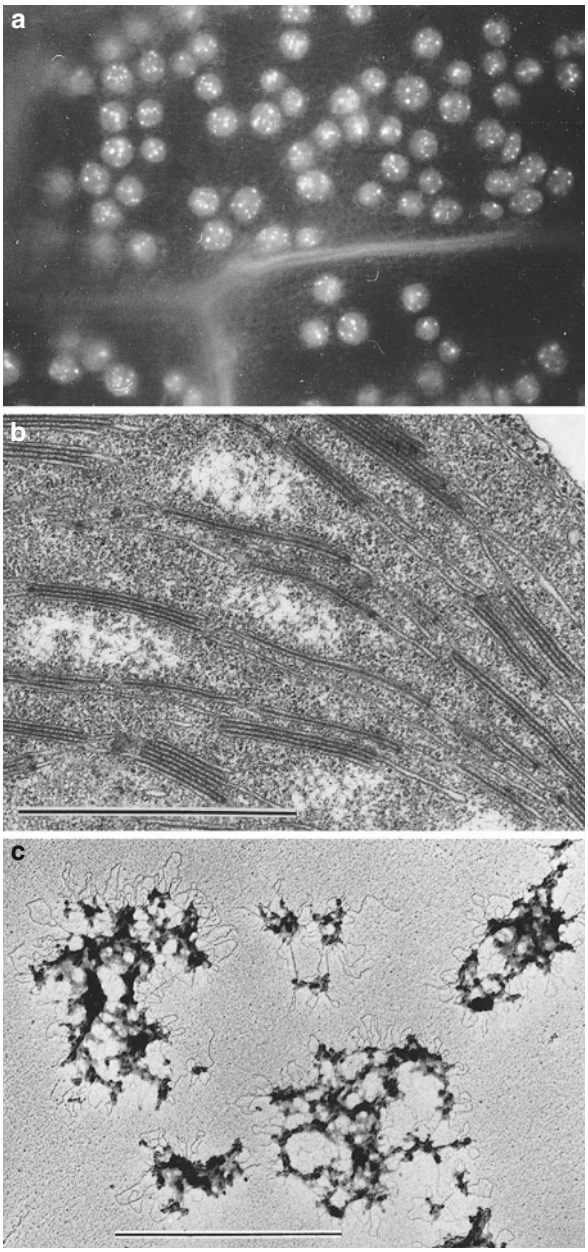

Fig. 2.85
Plastid nucleoids. (a) Chloroplasts in leaf cells of *Elodea canadensis* after fluorescence staining of the ptDNA with 4,5-diamidino-2-phenylindol (DAPI); each chloroplast contains several nucleoids, and each nucleoid contains several circular ptDNA molecules (×1,000). (b) Five nucleoids from the scattered region of a stroma matrix of a bean chloroplast. (c) Nucleoids isolated from spinach chloroplasts; ptDNA forms loops around scattered protein scaffolds. (b, c) *Scale bars* 1 μm (a Epifluorescence photograph from H. Dorle; b EM image from H. Falk; c preparation and EM image from P. Hansmann)

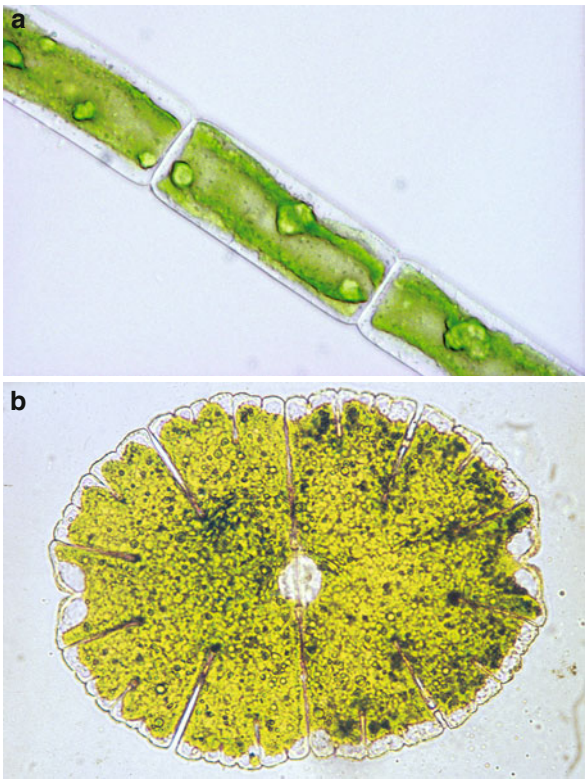

Fig. 2.86
Chloroplasts in cells of the alga *Mougeotia* (a; ×380) and *Micrasterias denticulata* (b; ×260)

particular plastid form in a cell reflects the function of that cell and is thus a consequence of tissue differentiation. For instance, the relatively small, often dividing **proplastids** reflect the high frequency of divisions in meristem cells.

Leucoplasts are typically found in cells that no longer divide and neither perform photosynthesis nor develop optical signals for animals. They can take on a storage function. Elaioplasts contain oil in numerous plastoglobuli, and proteinoplasts contain large protein crystals. Massive storage of starches is done by the nonpigmented **amyloplasts** in the corresponding storage tissues of, e.g., cereal grains and potato tubers.

Starch is the storage polysaccharide of green plants and many algae. It is the most important basic food source for humankind – wheat, rice, maize, and potatoes account for 70% of the sustenance for the world's peoples. Heterotrophic organisms (fungi, bacteria, animals) have glycogen instead of starch, which is deposited in the cytoplasm as flakes. Chemically, starch is, like glycogen, a homopolymer

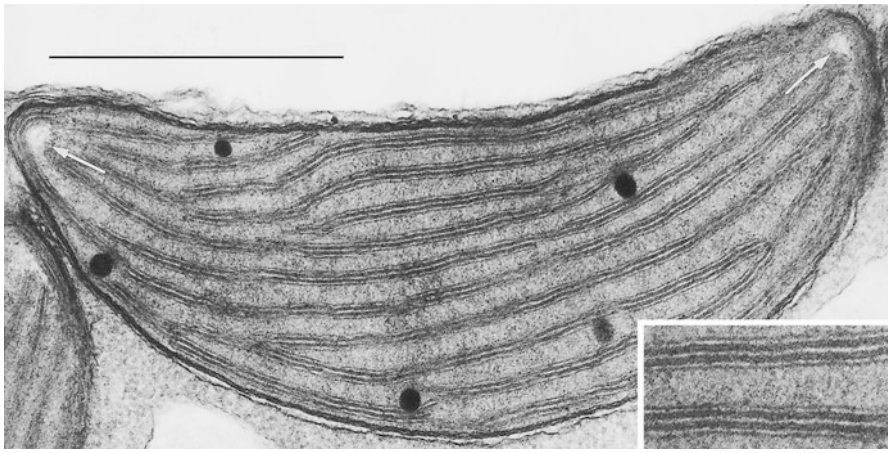

Fig. 2.87
Homogenic chloroplast of the alga *Tribonema viride* (see also Fig. 10.89e). The thylakoids, stacked in threes (section), run through the entire plastid; stroma thylakoids are not formed. ptDNA-containing regions are peripheral to the whole organelle (*arrow*). *Scale bar* 1 μm (EM image from H. Falk)

of α-glucose units (Fig. 1.20). When the spiral glucan chains are unbranched, then it is **amylose**; **amylopectin** has branched chains. (The glycogen molecule is even more branched.) Amylose and amylopectin are deposited in the form of very dense, birefringent **starch grains** inside the plastids or – as in some algae – in their immediate vicinity in the cytoplasm. The shape and the size of the starch grains in the storage tissues are generally species specific (*Fig. 2.89*).

The last steps in thylakoid genesis are light dependent in many (but not all) flowering plants. Under low-light conditions, the plastids of these plants become **etioplasts**, in which the building blocks of the thylakoid membrane or its precursors are accumulated in the form of a **prolamellar body** (*Fig. 2.90*). Etioplasts (French *étioler*, shrivel, stunted) are pigmented pale yellow by carotenoids. This is the coloration of shoots that, e.g., emerge from potato tubers sprouting in the dark.

Should green plants be exposed to permanent dark, the breakdown first results in stroma, then also grana thylakoid prolamellar bodies; the chloroplasts turn into etioplasts.

Chromoplasts have various ultrastructural types depending on their internal structures in which the lipophilic carotenoids (carotenes and xanthophylls; Fig. 5.45) are stored (*Fig. 2.91*).

- **Globular chromoplasts** occur most frequently, with numerous plastoglobuli in which the pigment molecules are concentrated.
- **Tubular chromoplasts** contain paracrystalline bundles of filaments (diameter, 20 nm) whose cross sections look like tubules under the electron microscope. However, these are actually nematic (threadlike) liquid crystals of the nonpolar pigments which are surrounded by a coat made up of amphipolar structural lipids and a structural protein of 32 kDa – fibrillin. Tubulose chromoplasts are strongly birefringent and can assume bizarre outlines.
- This is also true of **crystalloid chromoplasts**, in which β-carotene crystallizes out inside flat membrane sacks.
- The **membranous chromoplasts** are the least distributed. Their pigment molecules are built into membranes formed by the inner sheath membrane. They occur as concentric assemblages of many internested membrane cisternae. These membranes contain very little protein and are an example of lipid-dominated biomembranes.

The internal structures of chromoplasts are formed by molecular self-organization processes depending on the available types of molecules.

Chromoplasts, which often arise from juvenile chloroplasts or chloroplasts (unripe tomatoes, bell peppers, rose hips, etc., are green), are able to replicate by dividing by a sort of binary fission. In the process, the number of nucleoids per organelle is reduced, often to just one. At the same time, the plastid ribosomes are broken down and the ptDNAs are inactivated by compaction. Chromoplast-specific proteins, such as the fibrillin of tubulose chromoplasts, are always nuclear-encoded.

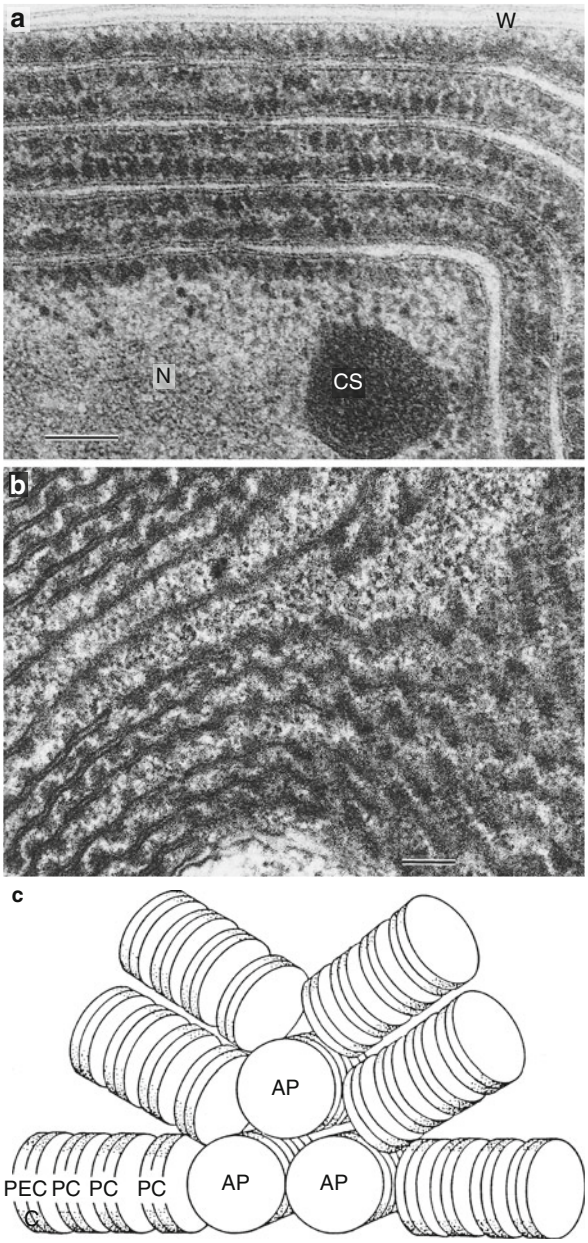

Fig. 2.88

Phycobilisomes. (a) In the cyanobacterium *Phormidium persicinum*. (b) In the red alga *Rhodella violacea*: *right* plane view; *left* in profile. (a, b) *Scale bars* 0.1 μm. (c) Molecular model of the semicircular disk-shaped phycobilisomes of red algae with a nuclear structure of allophycocyanin and radiating rows of phycocyanin and phycoerythrocyanin. For the role of phycobilisomes in photosynthesis, see Fig. 5.59. *AP* allophycocyanin, *CS* carboxysome, *PC* phycocyanin, *PEC* phycoerythrocyanin (see *Fig. 2.93a*), *N* DNA-containing centroplasm, *W* cell wall (EM images from W. Wehrmeyer)

The autumn foliage plastids – called **gerontoplasts** (Greek *géron*, venerable, geriatric) – have little in common with chromoplasts (*Table 2.4*). They are found in the cells of senescent leaves that are undergoing a massive substance breakdown.

2.3 Cell Structure in Prokaryotes

Prokaryotes are very heterogeneous whether viewed from an ecological, physiological, or structural point of view. The following is merely a first, short overview of the general characteristics of prokaryotic cell structures. It nevertheless highlights how great the differences are between prokaryotic cells and eukaryotic cells. There are no known intermediate forms between these two cell types among living organisms. Only related sequences of semantic molecules (e.g., DNA, RNA, proteins) indicate a shared phylogenetic origin of prokaryotes and eukaryotes.

The **fundamental differences between prokaryotes and eukaryotes** can already be observed externally by comparing the very disparate size of typical prokaryotic cells and eukaryotic cells (Fig. 1.1). A cell of the enteric bacterium *Escherichia coli* measures about 2–4 μm × 1 μm, which is a volume of about 2.5 μm^3. The plasma volume (without vacuoles) of the average eukaryotic cell is about 1,500–3,000 μm^3, about three orders of magnitude greater. This difference corresponds to the much smaller quantity of DNA in prokaryotic cells. Whereas the entire length of haploid human nuclear DNA measures about 1 m, that of *E. coli* is just over 1 mm. The miniaturization of prokaryotic cells has been accompanied by a shortening of the **generation turnover** in optimal conditions; in *E. coli*, this can be 20 min. Generally, eukaryotic cells in meristematic tissues do not divide more than once a day. A single bacterial cell can give rise to over one billion cells over a period of 10 h, which explains the enormous ecological significance of bacteria. There are no truly multicellular organisms among the prokaryotes.

The miniaturization of prokaryotic cells has also resulted in a particularly simple compartmentalization (*Fig. 2.92*). In most prokaryotic cells, the plasma membrane is the only biomembrane, and the cell is a single compartment. Intracellular nonplasmatic compartments are rarely formed in bacteria.

The so-called thylakoids of the cyanobacteria (*Fig. 2.93a*) are not components of their own membrane-surrounded plastids

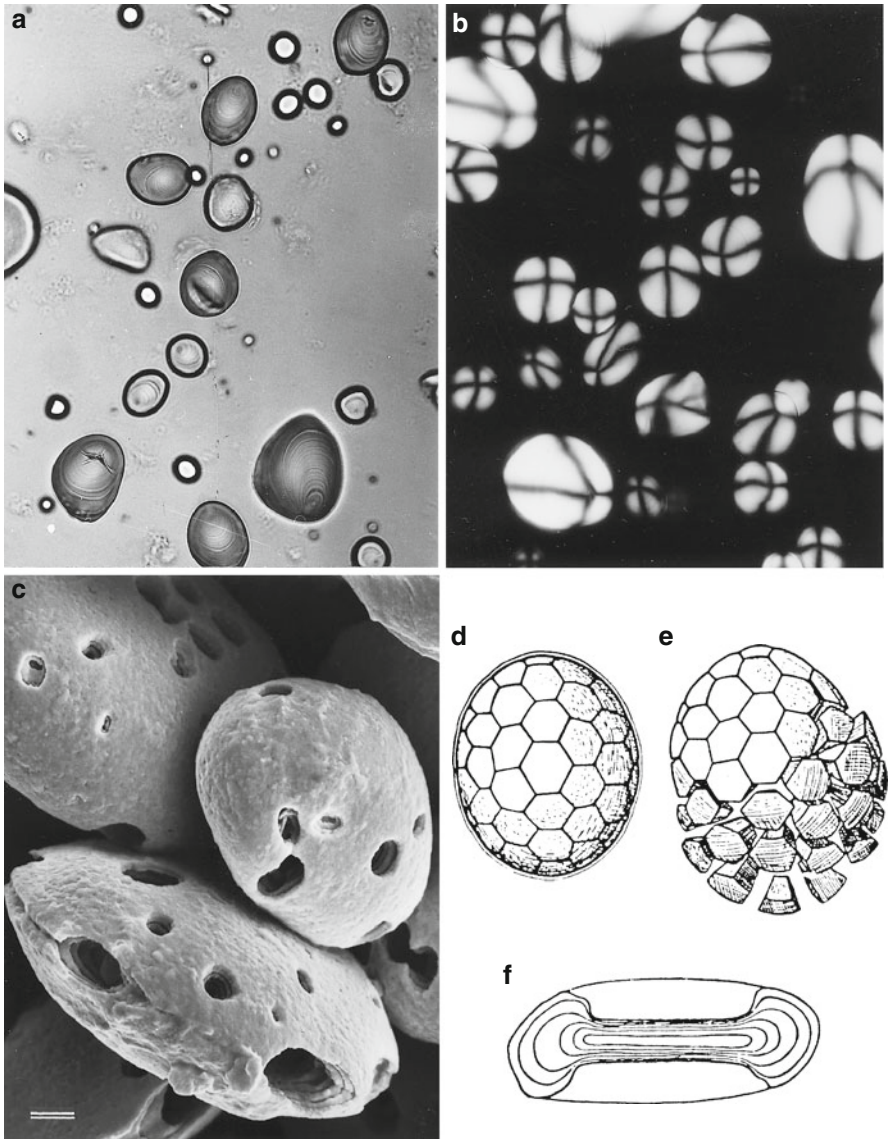

Fig. 2.89

Starch grains. (a, b) Potato starch (×330). (a) Layering distinct owing to the rhythmic fluctuations in synthesis conditions; starch grains generally grow from a common formation center ("hilum," in potato starch lies eccentrically) by the layered deposition of new material. (b) Under a polarization microscope, the starch grains are birefringent, whereby their concentric structure results in a characteristic dark cross. (c) Wheat starch after amylase treatment. The enzyme breaks down starch; the layered structure can be seen in the breakdown craters. *Scale bar* 1 μm. (d, e) Composite starch grains of oats. (f) Handle-shaped starch grain in an amyloplast from the latex of *Euphorbia splendens* (c Preparation by H.-C. Bartscherer, SEM image from Kontron, JEOL-EM JSM-840; d–f after D. von Denffer)

like in the algae and higher plants. They are instead flat double membranes located in the cytoplasm that are equipped with photosynthetic pigments that perform light reactions with water photolysis. They arise from the plasma membrane invaginations. Some bacteria have different forms of plasma membrane inclusion invaginations (*Fig. 2.93b, c*) which remain permanently attached to the plasma membrane. They are nevertheless called **intracytoplasmic membranes**. These membrane vesicles, pockets of tubes, also contain photosynthetic pigments.

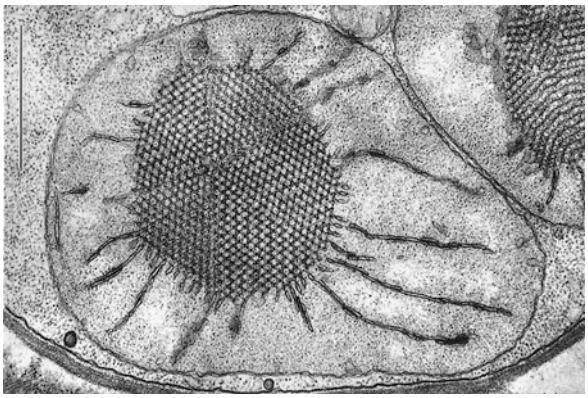

Fig. 2.90

Etioplast(s) in juvenile leaf cells of the green bean *Phaseolus vulgaris*. Individual thylakoids arise from paracrystalline prolamellar bodies. Plastid ribosomes are clearly smaller than cytoplasmic ribosomes; there are several nucleoids in the plastoplasm. *Scale bar* 1 μm (EM image from M. Wrischer)

The enormous diversity of the prokaryotes has by far not yet been completely researched. It is not only expressed in terms of their unique metabolic pathways, which are not found in eukaryotes. It is also documented in the great deviations from the typical characteristics described above. For example, bacterial cells can reach sizes similar to those of eukaryotic cells. Maximum values have been recorded for *Epulopiscium fishelsoni*, a Gram-positive enteric bacterium of tropical marine fish, with rod-shaped cells measuring 600 μm × 80 μm. The spherical sulfur bacterium *Thiomargarita namibiensis*, discovered in marine sediments off the coast of Namibia, has even larger cells, with diameters of up to 750 μm. It stores considerable quantities of sulfur and nitrate in its vacuolate cells. This bacterium forms chains of up to 50 cells. Similar cell colonies are typical of the cyanobacteria (Fig. 10.13), and in myxobacteria, it can even form complex, structured "fruit bodies."

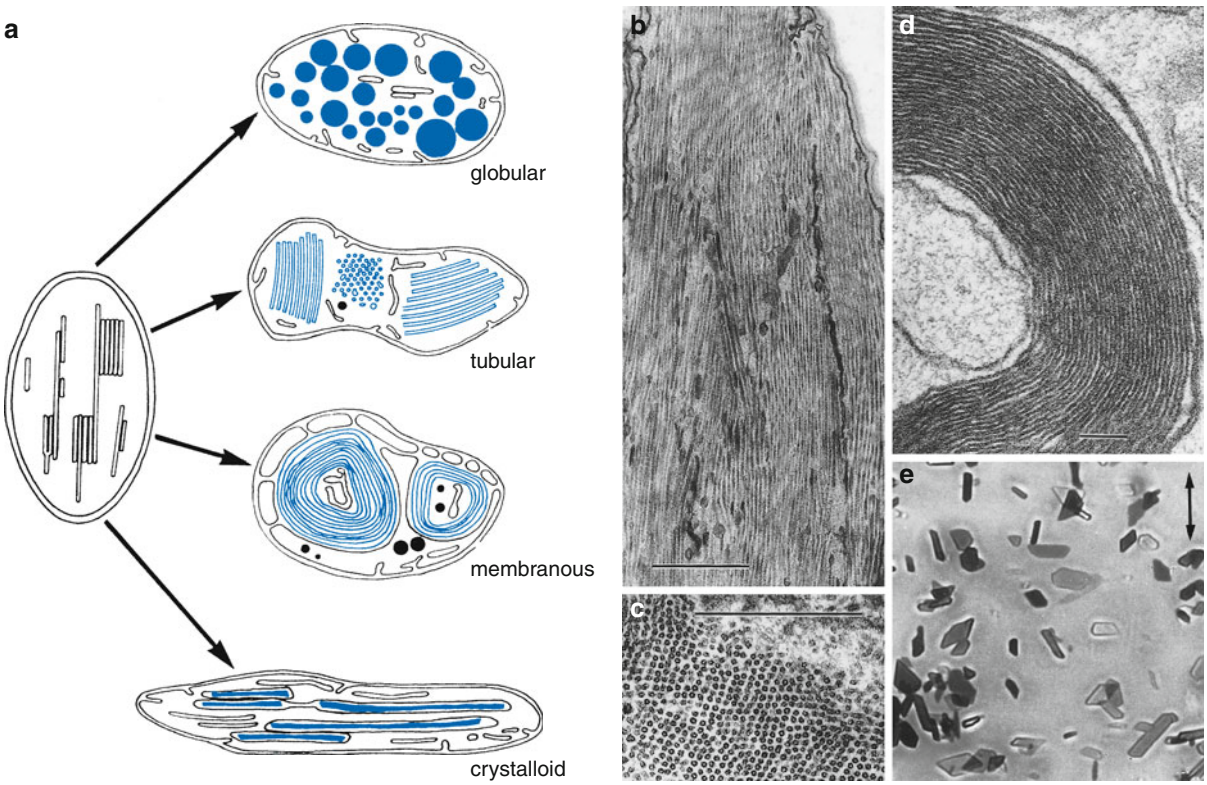

Fig. 2.91

Chromoplasts. (a) Types of ultrastructure; the development usually starts in young chloroplasts. (b, c) Tubulose chromoplasts lengthwise and transverse (haw, or petal of *Impatiens noli-tangere*; *scale bars* 0.5 μm). (d) Section of a membranous chromoplast of *Narcissus pseudonarcissus* (*scale bar* 0.1 μm). (e) Isolated crystalloid chromoplasts from the root of the cultivated carrot under polarized light (×750); the plate-shaped β-carotene crystals are dichroic, i.e., the light absorption depends on the direction of the light oscillation (tensor) (a After H. Mohr and P. Schopfer; e specimen from D. Kuhnen)

Table 2.4

Chromoplasts and gerontoplasts

Property	Chromoplasts	Gerontoplasts
Occurrence	Flowers, fruits	Autumn foliage
Function	Animals attraction	–
Origin from	Various plastid types via alteration or construction	Chloroplasts, by breakdown
Replication (division)	+	–
Ultrastructure type	Globulose, tubulose, membranose, crystallose	Always globulose
Novel synthesis of carotenoids	+	–
Cell status	Not senescent, anabolic	Senescent, catabolic

2.3.1 Reproduction and Genetic Apparatus

Prokaryote **DNA** is circular and does not exist in multiple various or linear pieces like in the chromosomes of the eukaryotes. Nevertheless, bacterial DNA rings are typically termed **bacterial chromosomes**. These chromosomes have a membrane attachment site and a single replication origin – they are replicons. The proportion of noncoding sequences is low. Despite their overall shortness (between 0.2 mm in mycoplasmas and 37 mm in some cyanobacteria), the DNA must be coiled up in a complex manner in order to fit the confines of the **nucleoid** (the DNA-bearing central zone in prokaryotic cells). The nucleoids are distinct from the ribosome-containing cytoplasm but are not divided from it with membranes or double membranes. Nucleolus-like structures are not found in the nucleoids. The centrally positioned nucleoid of the comparatively large cells (compared with the other prokaryotic cells) of the cyanobacteria ("blue-green algae") is visible under the light microscope. It is found in the centroplasm, which is surrounded by a peripheral chromatoplasm pigmented with "thylakoids" (*Fig. 2.93a*).

With the exception of the methanogenic archaea, there are no histones in nucleoids of prokaryotic cells. Neutralization and compaction of the genetic material is performed by other basic proteins, amines, and anorganic cations. The transcription and translation processes highlight the fact that nucleoids do not have a membranous

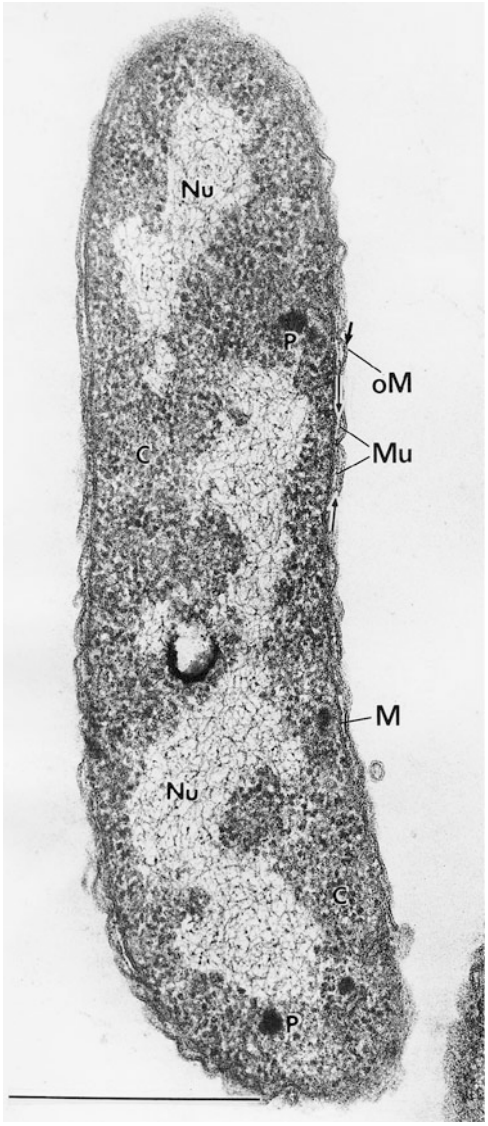

Fig. 2.92

Ultrastructure of a typical bacterial cell (Gram-negative) of *Rhodospirillum rubrum* (*scale bar* 0.5 μm). The irregularly formed nucleoid, in which the DNA strands are quite distinct, is surrounded by ribosome-rich cytoplasm with polyphosphate granules. The cell is separated from the cell wall by the plasma membrane. There is a thin "outer membrane" layer outside the thin murein sacculus (peptidoglycan layer, *thin arrow*), which is lacking in Gram-positive bacteria, whose murein sacculus is much thicker and has multiple layers (see *Fig. 2.97*). *C* cytoplasm, *M* plasma membrane, *Mu* murein sacculus, *Nu* nucleoid, *oM* outer membrane, *P* polyphosphate granules (Specimen from R. Ladwig, EM image from R. Marx)

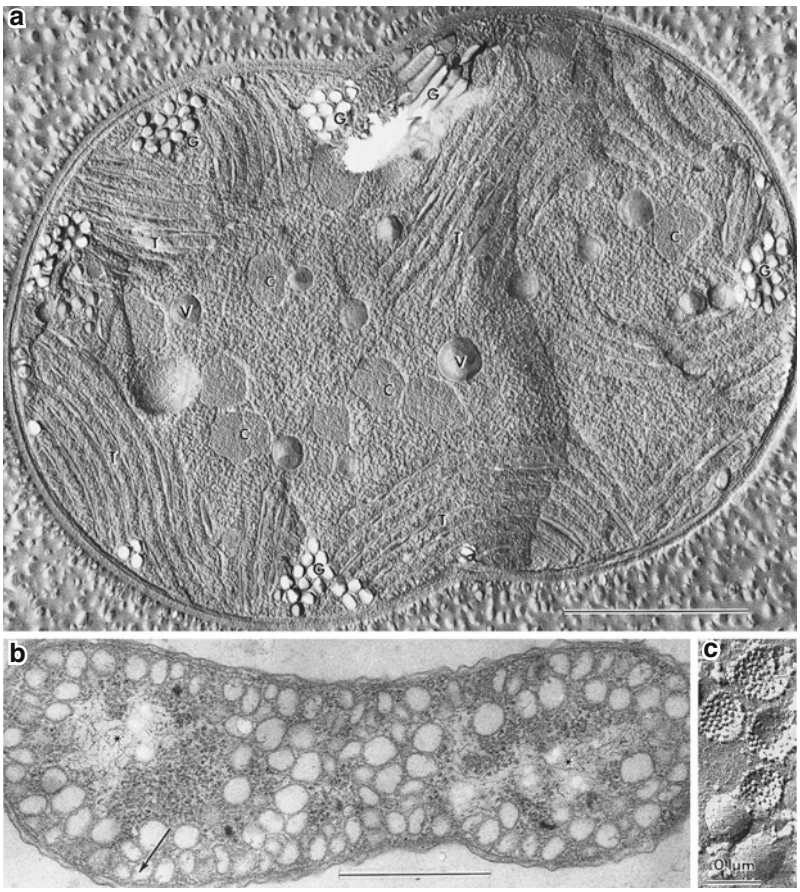

Fig. 2.93

Intracytoplasmic membranes (ICM) in prokaryotes. (a) *Microcystis aeruginosa*, a cyanobacterium (here after freeze fracture), contains several types of ICM: thylakoids, vacuoles with storage substances, carboxysomes as storage compartments for the photosynthetic enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (see Sect. 5.5.1); gas vacuoles (gas-filled hollow cylinders that enable the cell to float in water) are surrounded not by lipoprotein membranes but by protein coats that can be formed de novo in the plasma. The cell is about to divide. *Scale bar* 1 µm. (b) In the Gram-negative bacterium *Rhodospirillum rubrum*, an ICM of bubble-like chromatophores forms in light under aerobic conditions and together with the aid of bacteriochlorophyll perform photosynthesis – without splitting water; the chromatophores arise from cell membrane invaginations (*arrow*) and are always attached in part to the membrane and in part to each other. *Asterisks* nucleoids, *scale bar* 0.5 µm. (c) Equivalent chromatophores after freeze fracture of *Rhodobacter capsulatus*; extracellular fracture views seem smooth, plasma fracture views show lots of intramembrane particles. These are equivalent to protein pigment complexes for photosynthesis. *C* carboxysomes, *G* gas vacuoles, *T* thylakoids, *V* vacuoles (Preparations and EM images from J.R. Golecki)

boundary: before the transcription of a gene or a group of adjacent genes (an **operon**) is completed, translation starts at the first mRNA 5′-end to be synthesized. Processing of this RNA does not occur. Cotranscriptional translation happens on **70S ribosomes** (subunits 50S and 30S; *Fig. 2.42*), whose activity is inhibited by antibiotics different from those that work against the 80S ribosomes of eukaryotes (see Sect. 2.2.4). It is found that 70S ribosomes are smaller and simpler than 80S ribosomes.

There are no processes equivalent to mitosis and meiosis in prokaryotes. They have neither microtubules nor actin or myosin, and there is nothing immediately

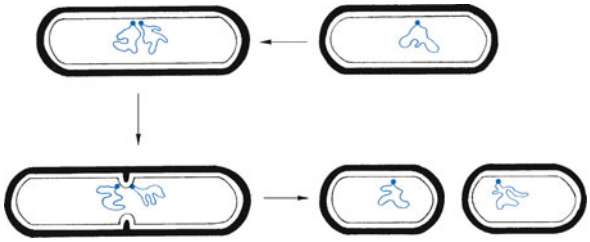

Fig. 2.94
Genome segregation and cell division in a bacterium. *Blue* circular DNA and attachment complexes on the cell membrane

comparable to the eukaryotic cell spindle apparatus. The distribution of the genetic material among the daughter cells happens after the doubling of the DNA ring molecule when the replication starting point and the membrane attachment sites are pushed as far apart as possible by membrane growth. A **septum** (cross-wall) begins to form between them (*Fig. 2.94*). In **cell division**, a contractile ring causes the cell plasma to undergo fission in the plane of the septum. The **protein FtsZ** plays a dominant role here. It is homologous to tubulin and under the right conditions will form filaments and ring structures.

Despite the lack of syngamy and meiosis, bacteria do undergo a sexual process, i.e., the transfer of genetic information from one cell to another and recombination (**parasexuality**). In particular, **plasmids** are transferred, which are (mostly) small ring-shaped DNA molecules that are able to autonomously replicate in the host cells. They do not have any genes for basic metabolic processes but rather carry adaptive genes that effect, e.g., antibiotic resistance (resistance genes on R-plasmids), or function in conjugation (F-plasmids) or code for toxins.

2.3.2 Bacterial Flagella

Many (eu)bacteria are flagellate, but their flagella are constructed completely differently from the complex flagella and cilia of the eukaryotes. **Bacterial flagella** (*Fig. 2.95*) are only 20-nm thick and are made of a uniform substance, **flagellin**. Bacterial flagella are spiral and stiff, unable to change shape. The base of the flagellum is anchored in the plasma membrane and cell wall with a storage structure made of four coaxial rings (*Fig. 2.96*). The flagellum itself is extracellular and, in contrast to the ten times thicker, flexible eukaryote flagellum, is not coated in a membrane sheath. During the

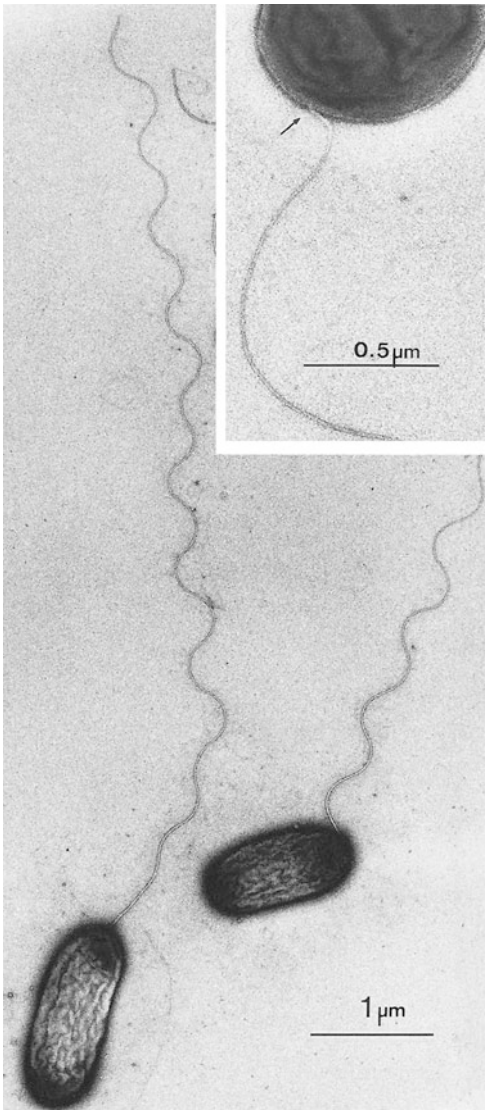

Fig. 2.95
Bacteria flagella (*Agrobacterium tumefaciens*, negative contrast). The *arrow* in the greatly enlarged split image shows the hook of the flagellum where the motor of the twisting motion is located (*Fig. 2.96*) (EM images from H. Falk)

constantly alternating forward and backward swimming of the bacterial cell, the whole flagellum twists in a clockwise or counterclockwise direction, like a ship's screw. The motor for this rotational movement is located at the base of the flagellum. It is driven not by ATP but directly by a proton gradient over the plasma membrane, which is leveled out by proton influx into the cell.

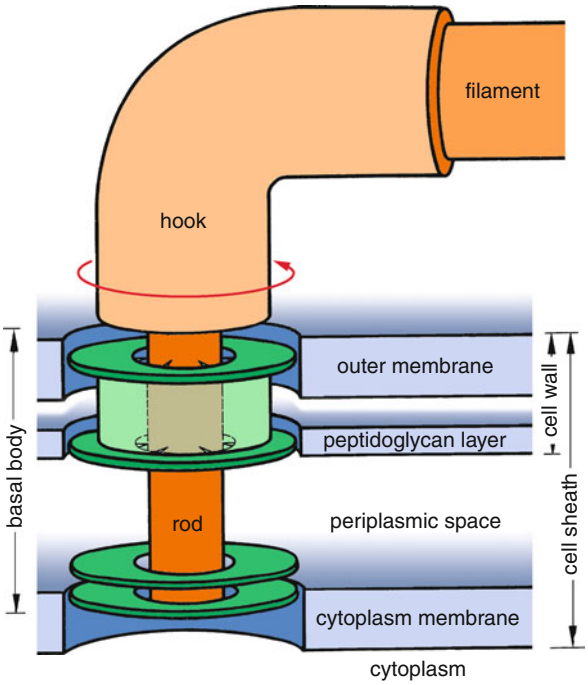

Fig. 2.96
The base of a flagellum of *Escherichia coli*. The four rings of the basal body that function as the driving mechanism have diameters of approximately 20 nm. The two outer ones are lacking in Gram-positive bacteria (After J. Adler)

2.3.3 Wall Structures

The cell walls of the prokaryotes can take on very different configurations. In the very small simple cells of the mycoplasma – they represent the lowest level of cellular organization – there are no cell walls at all. Most prokaryotic cells do have a cell wall, which not only protects the cell but also helps osmotic stabilization, gives the cell its shape, and controls contact with the environment. The wall functions as an outer (exo) skeleton. Prokaryotic cells whose walls have been removed artificially take on a spherical shape (spheroplasts and protoplasts); they are osmotically labile and only divide after the wall has been regenerated.

Figure 2.97 shows the layered construction of bacterial cell coats. (The cell walls of archaea are very different, even in their molecular building blocks.) A structure-defining component is the **peptidoglycan** or **murein coat**. It is made up of unbranched polysaccharide chains cross-linked with oligopeptide bridges. As the entire murein coat is in fact a single huge molecule, it is also called the **murein sacculus**. It can be enlarged by the local insertion of new building blocks and, in this way, accompanies cell growth without losing its support and protection functions. Peptidoglycan biosynthesis is blocked by penicillin. This antibiotic kills bacterial cells but not eukaryotic cells – peptidoglycans do not occur in eukaryotes.

Gram-positive and Gram-negative bacteria differ hugely in their cell wall structure. (**Gram stain** – gentian violet and iodine – can be washed out of Gram-negative bacteria using ethanol, but not out of Gram-positive bacteria.) In Gram-positive bacteria, the peptidoglycan coat is tough and composed of many murein layers. Gram-negative bacteria and cyanobacteria have a thin murein sacculus. A further characteristic layer is found outside the murein coat called the **outer membrane** because of its sectional appearance under the electron microscope. It resembles a biomembrane in terms of its molecular structure in that it is a lipid bilayer with a predominantly phospholipid inner layer. In contrast, the outer layer is formed from **lipopolysaccharides**, complex polymers with lipophilic fatty acids and outwardly oriented characteristic oligosaccharide and polysaccharide chains. Together, they form a hydrophilic protective layer around the prokaryotic cells through which lipophilic molecules are unable to permeate. Hydrophilic particles are allowed to pass through. Trimeric complexes of a transmembrane protein (**porin**) are located in the outer layer of the lipid bilayer and form about 1-nm-diameter hydrophilic pores. (The porins of the outer coat membrane of mitochondria and plastids have a comparable function but are not closely related to the comparatively much more heterogeneous bacterial porins.) The outer membrane is a cell wall layer, not a true biomembrane. In contrast to the biomembrane, it can also be formed de novo, e.g., it can be regenerated after the cell wall has been completely lost. It never makes contact with the cytoplasm and has no translocators for specific or active transport. The space between the cell membrane and the outer membrane is termed the periplasmic space.

Most prokaryotes are capable of forming **spores** with particularly strong and impermeable walls under unfavorable conditions.

2.4 The Endosymbiotic Theory and the Hydrogen Hypothesis

Plastids and mitochondria have a special position in eukaryotic cells: they are always separated from the cytoplasm by their double membrane coat and fuse – if at all – only with themselves. They have their own circular DNA and their own transcription and translation mechanisms

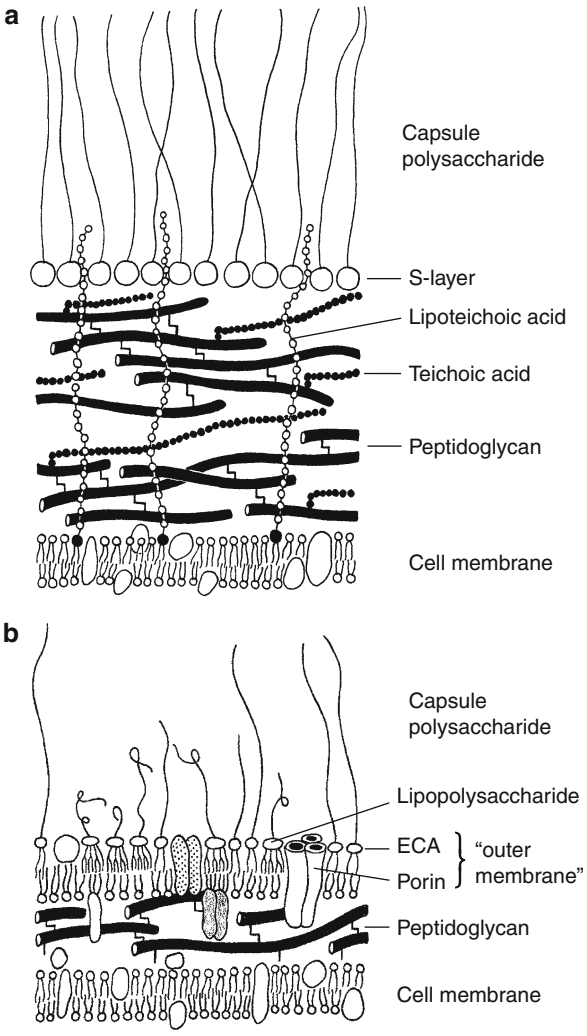

Fig. 2.97

Examples of bacterial cell walls. (a) The cell wall structure of a Gram-positive *Bacillus*; the cytoplasm membrane (cell membrane) is coated with multiple layers of peptidoglycans; teichoic acids run through the cell wall plane (linear polymers of glycerol phosphate or ribitol phosphate residues) that are covalently bonded to peptidoglycans; in contrast, the lipoteichoic acids are anchored in the cytoplasm membrane stretch perpendicularly to the wall. The entire wall complex is coated with the S layer ("surface" layer), to which the outwardly oriented chains of capsule polysaccharides are loosely bonded with covalent bonds. (b) Equivalent diagram for a Gram-negative bacterium, e.g., *Escherichia coli*. The peptidoglycan here is single-layered. The outer membrane is anchored in it by lipoprotein units (*gray*). It is permeated by trimeric porins and contains "outer-membrane" protein A (*dotted*) as an integral structural protein. The outer layer of the outer membrane is made of lipopolysaccharides with inwardly oriented fatty acids of lipid A and the outwardly spiraled polysaccharide chains (so-called O antigens) as well as amphipolar enterobacterial common antigen (*ECA*) units with longer stretched polysaccharide chains. Capsule polysaccharides ("K" antigens) are also anchored here (After U.J. Jurgens)

that have bacterial traits (see below). Even their mode of division is reminiscent of bacteria. The endosymbiotic theory postulates that given the features outlined, it is plausible that mitochondria and plastids are phylogenetically derived from bacteria and that in geologically early times were incorporated into primitive eukaryotic cells as intracellular symbionts (**endocytobionts**). The postulates of the endosymbiotic theory can be tested against actual endocytobioses.

2.4.1 Endocytobiosis

Endocytobionts can be found in many protists, animals, fungi, and plants. They assume the role of organelles in their host's cells. For example, bacteria of the genera *Rhizobium* and *Bradyrhizobium* living in the roots of legumes assimilate nitrogen and thus allow the host plant to be independent of nitrogen supplies in the soil or in fertilizer (see Sect. 8.2.1). In stone corals, endocytotic dinoflagellates (**zooxanthellae**; Fig. 10.81) can accelerate growth by up to ten times because of their photosynthetic activity. In amoebas, various ciliates, some fungi, and the freshwater polyp *Hydra* there are forms able to perform photosynthesis and which have become more or less photoautotrophic because of their endocytobiotic unicellular green algae (**zoochlorellae**). At any rate, the formation of stable endocytobioses is widespread and is also an ecologically significant phenomenon in living organisms (see Sect. 8.2).

Some endocytobionts can survive independently of their hosts. In other cases, the mutual dependency of the symbiotic partners is so pronounced that they only occur together in nature. Extreme examples of this are found among the unicellular **endocyanomes**, in which cyanobacteria live as permanent symbionts (*Fig. 2.98*). The endocytotic cyanobacteria function in the role of chloroplasts; they are called **cyanelles**. Cyanelles are unable to live outside their host. Their DNA only has half the total length and information capacity compared with the genome of free-living cyanobacteria. Most of the cyanelle-specific proteins are coded not in this DNA but rather in the nuclear DNA of the host cell. Thus, the

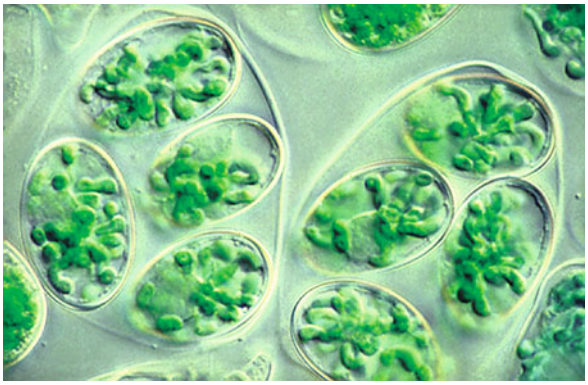

Fig. 2.98
Endocyanome. *Glaucocystis nostochinearum* with sausage-shaped cyanelles (×900) (LM image from P. Sitte)

cyanelles, which still have residual prokaryotic cell walls, have reached a situation identical to that of plastids, even from a genetic point of view.

2.4.2 Origin of Plastids and Mitochondria by Symbiogenesis

As already mentioned, the endosymbiotic theory is founded upon a series of special characteristics of plastids and mitochondria, which are also found in bacteria:

- Circular DNA without high numbers of repetitive sequences, with membrane attachment sites concentrated in nucleoids; no histones or nucleosomes.
- Replication is temporally independent of the S phase of the cell cycle.
- Sequence relatedness, e.g., of mitochondrial rRNAs with α-purple bacteria and of plastids with cyanobacteria.
- Only one rifamycin-sensitive RNA polymerase (there are three in the cell nucleus, which are variously sensitive to amanitine).
- Messenger RNA (mRNA) ends: no cap on the 5′ end, no poly-A extension at the 3′ end (see Sect. 6.2.2.2).
- Ribosomes that (among other things) are equivalent to the bacterial 70S type in terms of size and sensitivity to inhibitors.
- Translation starts with formyl methionine (not methionine as in the cytoplasmic 80S ribosomes).

There are other distinct parallels between organelles and bacteria. The inner mitochondrial membrane contains, e.g., cardiolipin, otherwise only found in bacteria, but lacks the sterol lipids typical of eukaryotic cell membranes (*Fig. 2.99*). The endosymbiotic theory postulates that incorporation of the endocytobionts must have happened by phagocytosis – the most widespread mechanism

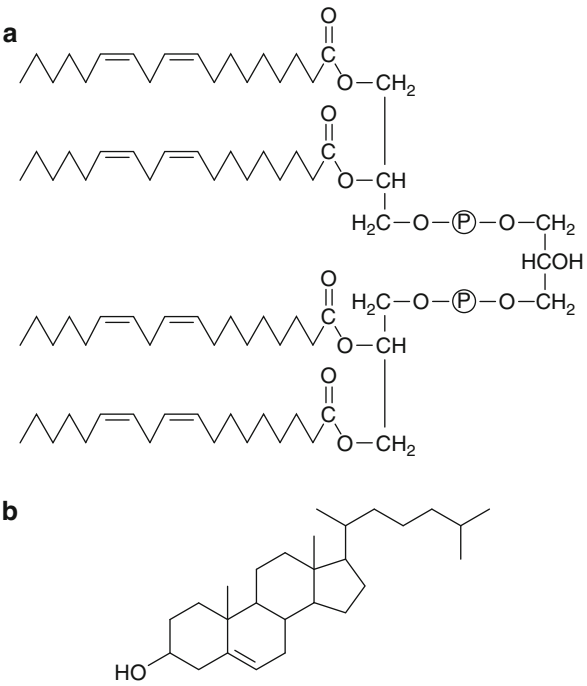

Fig. 2.99
Cardiolipin (a), a phospholipid, is widespread in bacteria but only occurs in the inner mitochondrial membranes of eukaryotic cells. Sterol lipids, e.g., cholesterol (b), are not present in the membranes of free-living prokaryotes or in inner mitochondrial membranes, but are frequent components in the membranes of eukaryotes

of nutrient uptake in protozoa (but also in the granulocytes and macrophages of mammals and humans) (*Fig. 2.100*). The well-known compartmentalization seen in plastids and mitochondria must occur by phagocytosis: phagocytized cells are surrounded by a double membrane within the scavenger cell although the inner membrane is the plasma membrane of the prey cell and the outer membrane is that of the phagosome (endosome) membrane, which in turn originated from the plasma membrane of the scavenger cell. Normally, after phagocytosis, the food particles are digested by lysosomes (*Fig. 2.55a*). This does not happen when endocytobioses are being established and the endocytosed unicells remain in the host cell as symbionts or parasites as documented by the examples among living organisms.

Therefore, an alternative hypothesis is currently being discussed, the so-called **hydrogen hypothesis**. Its fundamental tenet is that the primeval organisms did not exist as such in a phylogeny of the early evolution of life. Rather, the first eukaryotic cells were themselves products of a cellular symbiosis between methanogenic archaea and

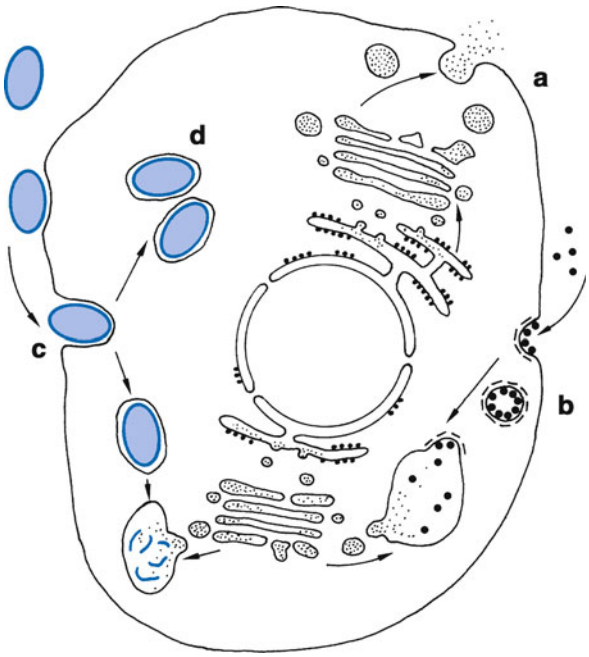

Fig. 2.100

Phagocytosis and endocytobiosis. A eukaryotic phagocyte (e.g., an amoeba) can, in addition to performing the endocytosis and exocytosis of molecular particles (*A*, *B*), also phagocytotically (*C*) engulf entire prey cells (*blue*) by folding the cell membrane into a feeding vacuole (a phagosome). When primary lysosomes are admitted, digestive vacuoles are formed. This is avoided when stable endocytobioses are formed (*D*): the prey cells survive in the host cell as symbionts (or parasites) and are able to reproduce within it

α-proteobacteria. Under conditions of oxygen shortage, the bacteria form hydrogen, which the archaea require for methane synthesis. This symbiosis would have then enabled the archaea to become independent of abiotic hydrogen sources. The archaea so completely incorporated their partners that they then developed either into **hydrogenosomes** (DNA-free mitochondria equivalents in the anaerobic eukaryotic unicells) or, depending on oxygen availability, into mitochondria. According to this hypothesis, the primitive eukaryotic cells already contained α-proteobacteria and did not need to subsequently incorporate them by phagocytosis, contrary to what the endosymbiotic theory postulates. The hydrogen hypothesis is supported by the fact that the methanogenic archaea have histones and form nucleosomes, a feature otherwise restricted to the eukaryotes.

The most important general conclusion that can be drawn from the endosymbiotic theory is that novel organisms do not have to arise via mutation, genetic recombination, or horizontal gene transfer but can also arise from the development of stable intracellular symbioses. These types of **intertaxon combinations**, creating novel superorganisms, are chimeras from both a chemical and a genetic point of view. (In biology, a chimera is an organism that is not genetically uniform.) Modern eukaryotic cells are chimeric **mosaic cells**, made up of cells from different kingdoms. During **symbiogenesis** – the long process of coevolution between the host and endocytobionts – the symbionts have gradually turned into the organelles, as observed in modern eukaryotic cells. The changes pertained to wall loss; coordination of reproduction, and stricter organization according to the needs of the host cell; development of translocator systems of the coat membranes for substance exchange or the ability to transfer ATP or triose phosphate outward; and finally the relocation of genetic information from the symbiont/organelle to the nucleus of the host cell combined with the specific import of proteins and tRNAs from the cytoplasm into the organelles.

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