

MINISTRY OF SCIENCE AND HIGHER EDUCATION OF THE RUSSIAN FEDERATION

National Research Lobachevsky State University of Nizhny Novgorod

E.B. Romanova

C Y T O L O G Y

Manual

Recommended by the Academic Council of the UNN Institute of Biology and Biomedicine for students in the areas of studies 06.03.01 «Biology», 05.03.06 «Ecology and environmental management»; specialties: 30.05.01 «Medical Biochemistry», 30.20.02 «Medical Biophysics», 30.05.03 «Medical Cybernetics», 31.05.03 «Dentistry», 31.05.01 «General Medicine»

Nizhny Novgorod
2020

UDC 576.3
LBC E 05
R-69

Reviewers:

A.I. Dmitriev, Doctor of Sciences (Biology) (NSPU);
A.V. Deryugina, Doctor of Sciences (Biology) (UNN)

Romanova, E.B.

R-69 **Cytology:** manual / E.B. Romanova. Russian text translated by A.G. Lubavsky. –
Nizhny Novgorod: Nizhny Novgorod State University Press, 2020. – 128 p.
ISBN 978-5-91326-601-9

The manual presents modern data on the structure and functions of prokaryotic and eukaryotic cells, examines basic organoids, their interrelation in realization of cellular functions and intracellular processes. Each of the manual's ten chapters includes a theoretical part with illustrations and descriptions of cell structures. Control tests and topics for independent work at the end of each chapter are intended to help students check their knowledge and improve their level of understanding the subject. The glossary includes 100 annotated terms.

Readership: students in the areas of studies 06.03.01 «Biology», 05.03.06 «Ecology and environmental management»; Specialties: 30.05.01 «Medical Biochemistry», 30.20.02 «Medical Biophysics», 30.05.03 «Medical Cybernetics», 31.05.03 «Dentistry», 31.05.01 «General Medicine».

ISBN 978-5-91326-601-9

UDC 576.3
LBC E 05

© E.B. Romanova, 2020
© Lobachevsky State University of Nizhny Novgorod, 2020

CONTENTS

INTRODUCTION	5
Chapter 1. Cytology: the cell science.....	6
1.1. History of development and methods of cytology.....	6
1.2. Design and principle of operation of the light microscope.....	9
1.3. Types of cell organization.....	16
Control tests.....	20
Topics for independent work	21
Chapter 2: Chemical composition of the cell.....	22
2.1. Inorganic substances	22
2.2. Organic compounds	25
Control tests	30
Topics for independent work	30
Chapter 3. Prokaryotic cell biology	32
3.1. Features of organization and diversity of prokaryotes.....	32
3.2. Features of prokaryote cell cycle and surface apparatus	34
Control tests.....	37
Topics for independent work	37
Chapter 4. Cell membranes.....	38
4.1. Lipid bilayer, the structural base of membranes.....	38
4.2. Asymmetry of cell membranes	41
4.3. Membrane proteins.....	42
Control tests.....	48
Topics for independent work	49
Chapter 5. Cytoskeleton and membrane contact function	50
5.1. Actin filament system	50
5.2. Tubulin – microtubule system	55
5.3. Intermediate filament system	57
Control tests.....	58
Topics for independent work	59
Chapter 6. Vacuolar system	60
6.1. Endoplasmic reticulum.....	60
6.2. Golgi complex	64
6.3. Lysosomes.....	68
6.4. Peroxisomes.....	69
Control tests.	71
Topics for independent work	71
Chapter 7. Mitochondria	72
7.1. Structure and function of mitochondria	72
7.2. Localization of the main links of oxidative phosphorylation in the membranes of mitochondria	73

7.3. Mechanism of oxidative phosphorylation.....	75
Control tests	77
Topics for independent work	77
Chapter 8. The centrosome	78
8.1. Centrioles	78
8.2. Centriole replication.....	79
Control tests	81
Topics for independent work	82
Chapter 9. Eukaryotic cell nucleus.....	83
9.1. Chromatin.....	84
9.2. Histones, basic chromatin proteins	84
9.3. Packaging of DNA molecules.....	85
9.4. Surface apparatus	91
9.5. Nucleolus.....	94
Control tests.....	98
Topics for independent work	99
Chapter 10. The cell cycle	100
10.1. Interphase	102
10.2. Mitosis.	102
10.3. Cytokinesis	107
10.4. Cell cycle regulation	108
Control tests.....	114
Topics for independent work	114
GLOSSARY	115
INFORMATION RESOURCES FOR THE SUBJECT	
«CYTOLOGY»	127

INTRODUCTION

The aim of this manual is to familiarize students with the basic principles of modern research methods, with structural and functional features of prokaryotic and eukaryotic cells. It also serves to make up for the lack of an appropriate textbook and instructional materials on the subject «Cytology».

The manual consists of ten chapters. Each chapter has a theoretical part and a description of cell structures and intracellular processes and also contains compulsory control test tasks to be performed by students.

Of special importance is students' independent study of individual sections and topics of the subject. This approach develops students' initiative, their desire to acquire more knowledge and to develop skills and abilities required for mastering the methods and techniques in their professional field. The whole system of individual independent work should be aimed at acquiring the conceptual framework, since one of the most important tasks in the training of modern competent specialists is to teach them professional terminology and the way to use it in a competent manner. A glossary at the end of the manual will help students to achieve the learning objectives.

Note-taking is one of the main forms of students' independent work, which requires extensive use of educational literature in addition to lecture notes. The study of recommended literature should begin with textbooks and manuals. After that, students should move on to scientific monographs and periodicals.

This manual is not intended to replace the material of the lectures and the textbook. It can be considered as an option to systematize the modern framework of cytological knowledge with the aim to facilitate the understanding of the material and to optimize the learning process.

Author

CYTOLOGY: THE CELL SCIENCE

1.1. History of development and methods of cytology

At the heart of the diversity of living beings in the biosphere is a cell as a universal structural and functional unit of all forms of life. The cell is a membrane-bound ordered structured system of biopolymers (proteins, nucleic acids) and their macromolecular complexes participating in an integral set of metabolic and energy processes that provide for support and reproduction of the system as a whole.

The science of the cell – **cytology** (from Greek *kytos* – cell) became a separate branch of biological science more than 100 years ago. The objects of study of cytology are cells of multicellular and unicellular organisms, plant cells, nuclear-cytoplasmic complexes that are not divided into cells, as well as bacteria. The original concept of «cell» was introduced by the English researcher Robert Hooke. One of Hooke's most notable discoveries came in 1665 when he looked at a sliver of cork through a microscope lens and discovered what he called «cells». Hooke described them in his main work, «Micrographia» (**fig. 1.1**).

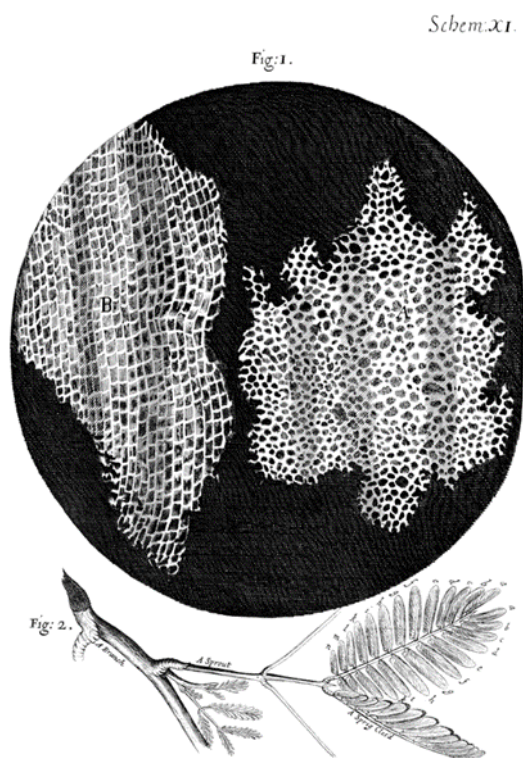


Figure 1.1. Drawing of the first cells made by Robert Hooke (1635-1703) (Micrographia, 1665) (<https://ru.wikipedia.org/wiki/Micrographia#/media/%D0%A4%D0%B0%D0%B9%D0%BB:RobertHookeMicrographia1665.jpg>)

The development of cytology is closely connected with the creation of optical lenses and a complex microscope constructed using such lenses. There is evidence in the literature that the first microscope in 1590 was designed by the Dutch spectacle maker Hans Janssen and his son Zacharias. Another contender for the title of inventor of the microscope was Galileo Galilei. He developed in 1609 a composite microscope with convex and concave lenses. Ten years later, C. Drebbel invented a new type of microscope with two convex lenses. Christiaan Huygens, another Dutchman, invented a simple two-lens eyepiece system at the end of the 17th century which was adjustable and therefore this system made a huge step forward in the history of microscopes. Huygens' eyepieces are still produced today, but their field of view is not wide enough.

Antonie van Leeuwenhoek was a great master in the field of microscopy. Van Leeuwenhoek's handmade microscopes were relatively small pieces with one very strong lens. It was Leeuwenhoek who first drew the attention of biologists to the microscope. In 1680, he discovered the world of unicellular organisms and was the first to see red blood cells in the microscope. However, the idea of cells at that time was still very primitive. Cells were seen as «voids» or «air spaces within a shell». The shell was considered the main structure of cell organization.

Looking at cells in the light microscope. A typical animal cell is 10–20 μm in diameter, which is about one-fifth the size of the smallest particle visible to the naked eye. Only after good light microscopes became available in the early part of the nineteenth century did Schleiden and Schwann propose that all plant and animal tissues were aggregates of individual cells. Their discovery in 1838, known as the **cell doctrine**, marks the formal birth of cell biology.

Animal cells are not only tiny, but they are also colorless and translucent. Consequently, the discovery of their main internal features depended on the development, in the latter part of the nineteenth century, of a variety of stains that provided sufficient contrast to make those features visible. Similarly, the far more powerful electron microscope introduced in the early 1940s required the development of new techniques for preserving and staining cells before the full complexities of their internal fine structure could begin to emerge. To this day, microscopy relies as much on techniques for preparing the specimen as on the performance of the microscope itself. In the following discussions, we therefore consider both instruments and specimen preparation, beginning with the light microscope.

The formulation of particular provisions of cell theory was preceded by a rather long (more than three hundred years) period of accumulation of observations over the structure of various unicellular and multicellular plant and animal organisms. Significant progress in the study of cells is associated with the development of microscopy in the 19th century. By that time, the ideas about the structure of cells had changed: the main role in the organization of the cell was attributed to its content, the protoplasm (Purkinje, 1830). The constant component of the cell – its nucleus – was discovered in protoplasm (Brown, 1833).

Theodor Schwann was the first scientist to conclude that all animal tissues are made up of microscopic elements, i.e. cells. To make sure that all living organisms consist of cells, Schwann invited his friend, the German botanist Matthias Schleiden to cooperate. It took them a very long time to check the correctness of their assumptions using a microscope. When all doubts were removed, Schwann published his landmark work, *Microscopical Researches into the Accordance in the Structure and Growth of Animals and Plants* in 1839. This was how one of the most important biological theories, called cell theory, was developed. Cell theory gives a generalized idea about the structure of cells as units of living, about their reproduction and role in the formation of multicellular organisms. Cytology was further developed in the works of Rudolf Virchow (1821–1902). Virchow expressed his beliefs (1858) in the form of the following maxim: every cell arises from another cell.

The main tenets of cell theory have retained their significance to this day, although more than a hundred and fifty years have seen new information about the structure, vital functions and development of cells.

At present, cell theory postulates:

1. The cell is an elementary unit of all living beings.
2. The cell is a single system comprising many elements that are regularly connected with each other and constitute a definite integral formation consisting of conjugated functional units known as organelles or organoids.
3. Cells are similar (homologous) in terms of their structure and basic properties.
4. Cells increase in number by division of the original cell, which is preceded by the reproduction of its genetic material (DNA reduplication).
5. A multicellular organism is a new system, a complex ensemble of many cells combined and integrated into systems of tissues and organs interrelated through humoral and nervous chemical factors (molecular regulation).
6. Cells of multicellular organisms are totipotent (i.e., they have the genetic potential to be any cell of a given organism) and are equivalent in their genetic information, but differ from each other in different expression (work) of different genes, which leads to their morphological and functional diversity based on the phenomenon of differentiation.

The emergence of the cell theory has become the most important event in biology, one of the decisive proofs of the unity of all the elements of the living nature. Scientists around the world felt the need for a more extensive in-depth study of the cell structure.

In 1884, J.B. Carnoy published his book «Cell Biology», which summed up generalized information about cell structure for the first time. In 1892, the first edition of O. Hertwig's remarkable work «The Cell and the Tissues» was published. A Russian translation of this book appeared in 1904.

The period at the end of the 19th century and the early 20th century was marked by further improvement of microscopes and the search for new research methods. It was already obvious that cells have an unusually complex structure.

Modern cytology is the physiology of the cell. Over the past 50 years, cytology has turned from descriptive-morphological to experimental science, which aims to study the physiology of the cell, its basic vital functions and properties, its biology. Cytology fruitfully combines both morphological and molecular-biological approaches, and the subject of its study is the cell, which has its own laws of organization and functioning.

1.2. Design and principle of operation of the light microscope

Biological (light) microscope is the main instrument used in biology to study cells. A light microscope is an optical system consisting of a condenser, a lens and an eyepiece. This system is precisely centered and mounted on a mechanical part (base with an arm). The light beam from the light source is collected in the condenser and directed to the object. After passing through the object, the light rays enter the lens system where the primary image is built. The image is magnified with the help of eyepiece lenses (**fig. 1.2**).

Objects of light microscopy are specimens that can be seen in the passing light.

Let us consider the main parts of a biological microscope (**fig. 1.3**). The key part of a microscope is its optical assembly. It consists of a lighting system (condenser and illuminator), objectives mounted in the revolving nosepiece, and eyepieces. All parts of the optical assembly are precisely centered.

The objective is a multi-lens system, the key component of a microscope's optical assembly that determines its basic capabilities. The main parameters of the objective are set by a universally recognized international standard that defines the barrel length (160 mm), the objective height (45 mm) and the color coding bars around the objective. There are so-called «dry» and immersion objectives. Immersion objectives have a higher numerical aperture (1.25–1.4)

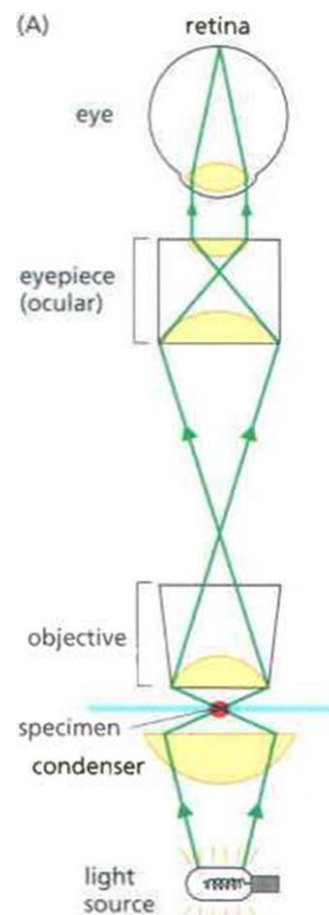


Figure 1.2. A light microscope Diagram showing the light path in a compound microscope. Light is focused on the specimen by lenses in the condenser. A combination of objective lenses and eyepiece lenses are arranged to focus an image of the illuminated specimen in the eye

than dry objectives because the refractive index of liquids is greater than 1.0; water has a refractive index of 1.33 and immersion oil has a refractive index of 1.52. It should be noted that the refractive index of immersion oil is close to that of glass ($n=1.52$), so when using oil, a homogeneous system is created which reduces the dispersion of rays and improves image clarity. Oil-immersion objectives are marked by letters and a dark or white color ring. 1.

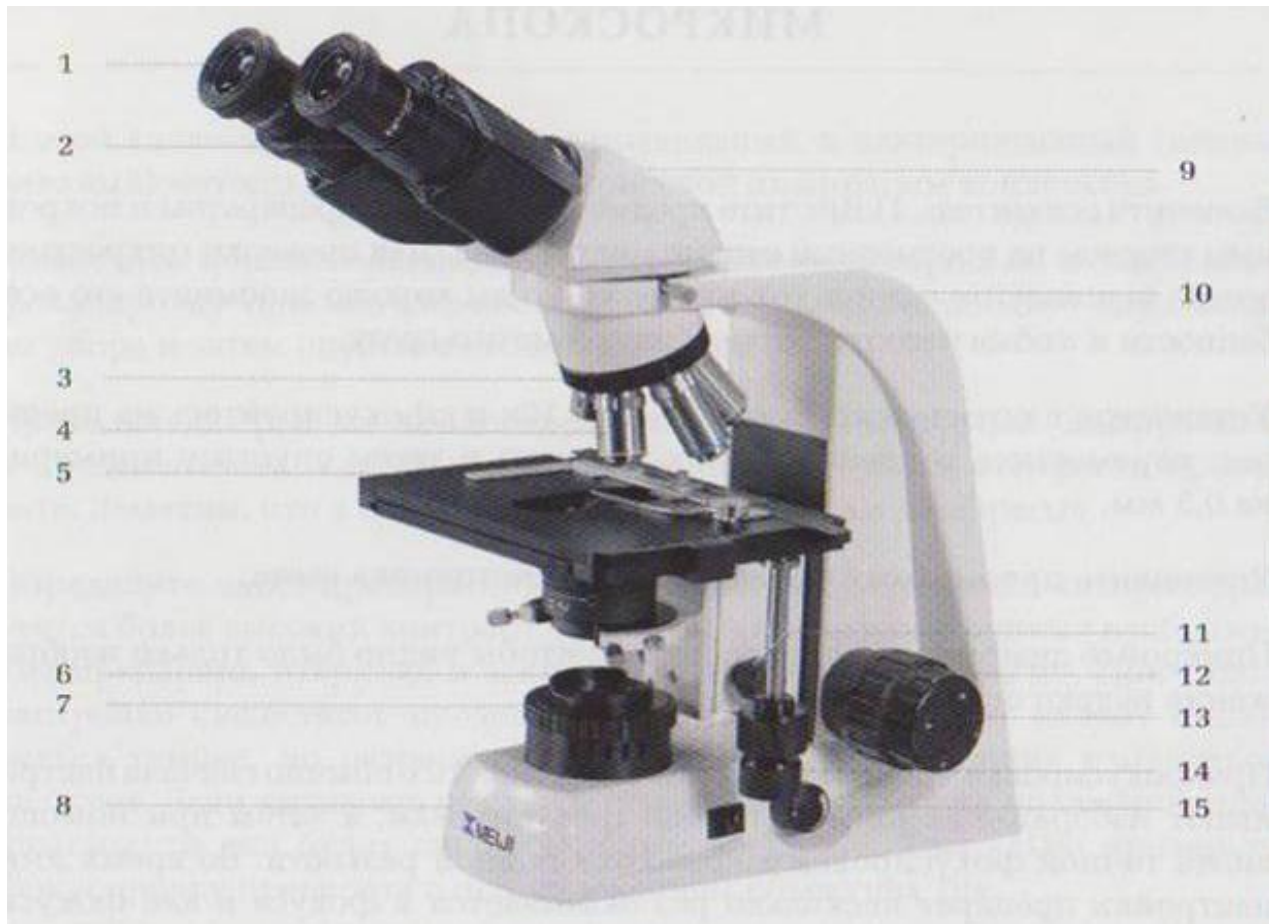


Figure 1.3. Main parts of a biological microscope

1 – eyepiece; 2 – dioptic device;
3 – revolver; 4 – objectives; 5 – stage;
6 – illuminator; 7 – illuminator
diaphragm; 8 – microscope stand;

9 – binocular attachment; 10 – microscope arm;
11 – condenser movement control; 12 – coarse focus
knob; 13 – fine focus knob; 14 – coaxial knobs for
stage movement; 15 – brightness regulator;
16 – condenser; 17 – condenser alignment knobs;
18 – condenser aperture diaphragm;
19 – filter holder

(https://books.google.ru/books?id=a6yKDQAAQBAJ&pg=PT104&lpg=PT104&dq=%22immersion+objectives+are+marked+%22&source=bl&ots=wPvoHGYsf_&sig=ACfU3U0daT79iTEFHn9hIBmloj19WSJpvw&hl=en&sa=X&ved=2ahUKEwiPgcuosfoAhUPw8QBHUa4CRUQ6AEwBnoECAoQOg#v=onepage&q=%22immersion%20objectives%20are%20marked%20%22&f=false)

Each objective can be described using the following characteristics:

1. Curvature or flatness of the field of view.
2. Magnification.
3. Resolving power.
4. Chromatic correction.

Field curvature of the objective determines the part of the field of view that is in focus. According to this characteristic, objectives are divided into three main categories. Achromatic objectives have a flat field in the central 2/3 of the image. With semi-planar objectives, 80% of the field of view is in focus. Planar objectives provide almost 100% of the field of view in focus.

Magnification is indicated on the barrel of the objective. Low magnification objectives (x8 and x10) have the maximum working distance and a wide field of view, so the study of the specimen begins at a low magnification. High magnification objectives (x20; x40; x100) have a shorter focal length.

Resolving power of an objective lens is measured by its ability to differentiate two adjacent lines or points in an object. The greater the resolving power, the smaller the minimum distance between two lines or points that can still be distinguished.

The resolving power (α) of an objective depends on its numerical aperture (NA) and the wavelength of light (λ) used to illuminate the specimen and is determined by the formula:

$$\alpha = \frac{\lambda}{NA}$$

When using side lighting of the object (Tyndall effect: dust particles can be seen in the light beam in a dark room with side lighting), the resolution is doubled:

$$\alpha = \frac{\lambda}{2NA}$$

Numerical aperture (NA) characterizes the light-gathering capacity of an objective and is determined by the formula:

$$NA = n \cdot \sin \beta$$

where n is the index of refraction of the medium between the objective front lens and the cover glass;

β is the half aperture angle of the beam cone coming out of the object point and bounded by the entrance pupil of the lens. The value of the numerical aperture is constant for the objective and is engraved on its barrel.

The higher the numerical aperture value, the better the resolution of the objective. The resolution of the objective also depends on the wavelength of light that illuminates the object. Typically, light microscopes use light sources in the visible region of the spectrum (0.35–0.75 μm), so the maximum resolution of the microscope in this case may not exceed 0.2–0.35 μm . The light microscope, as an

auxiliary device to our eye, increases its resolution by about 1500–2000 times (the naked human eye has a resolution of about 0.1 mm, or 100 μm). Therefore, when using the visible light, the resolution limit of the light microscope is 0.2 μm .

Chromatic correction. Objective lenses may introduce some distortions (aberrations). Chromatic aberration is caused by the fact that the violet part of the light spectrum is refracted more than the red part, so the image created by rays of one wavelength does not coincide with the image created by rays of another wavelength, as a result the image can have color fringes. In terms of chromatic correction capacity (correction of chromatic aberration), objectives are classified into achromats, semi-apochromats (fluorites) and apochromats. In achromats, chromatic aberration is corrected for the two outermost wavelengths (red and violet), i.e., the focus for these beams is brought to the same point. In fluorite objectives, special glass is used which brings all areas of the spectrum closer to the same focus. Apochromats provide correction for aberration of the three basic colors (red, green, blue), bringing all other areas of the spectrum to almost the same focus. Such objectives can offer a much better image quality.

A very powerful analytical tool for studying living cells is **fluorescent microscopy** that involves the use of special dyes. This method is based on the ability of some substances to glow when they absorb light energy (fluorescence). The fluorescence spectrum is always shifted towards long wavelengths with respect to the radiation that excites fluorescence, that is why objects are examined in fluorescence microscopy in the short wavelength zone. Many dyes selectively bind to certain structures of the cell, causing them to emit light (*fig. 1.4*).

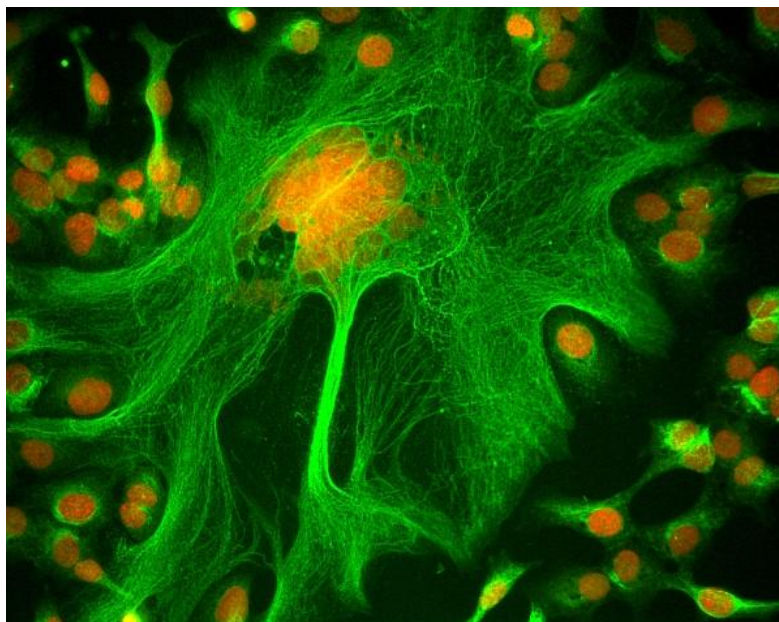


Figure 1.4. Mouse fibroblasts

(<https://fainaidea.com/nauka/materialy/atomnaya-mikroskopiya-i-voda-klyuchevye-factory-uluchsheniya-hraniya-informatsii-144309.html>)

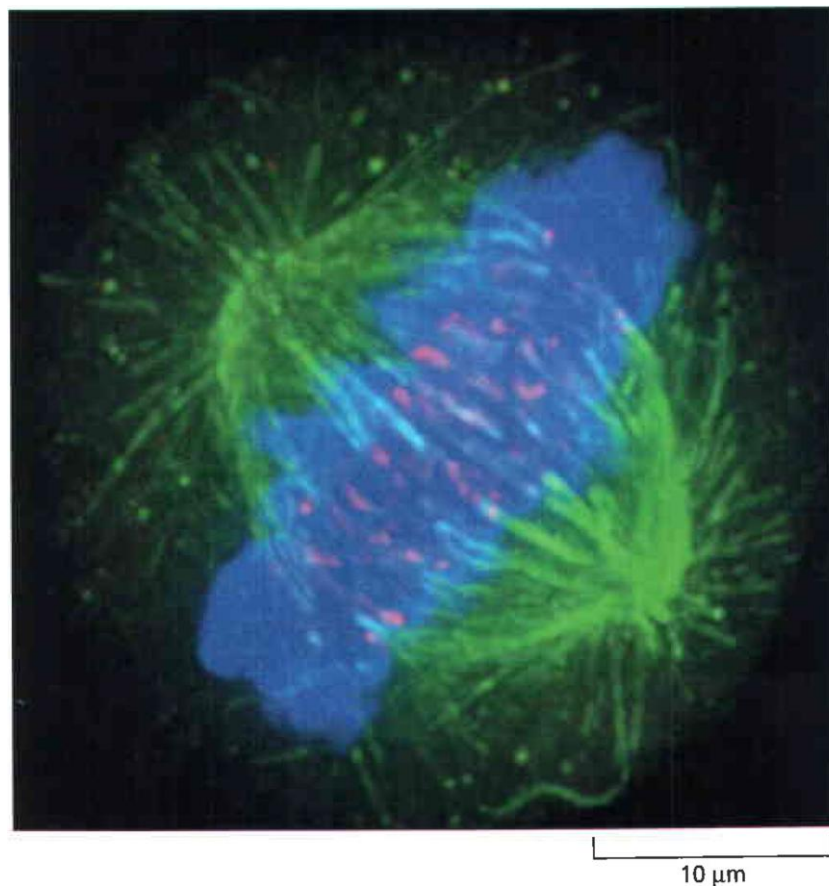


Figure 1.5. Multiple-fluorescent-probe microscopy (Alberts et al., 2008, p. 587)

In this composite micrograph of a cell in mitosis, three different fluorescent probes have been used to stain three different cellular components. The spindle microtubules are revealed with a *green* fluorescent antibody, centromeres with a *red* fluorescent antibody and the DNA of the condensed chromosomes with the *blue* fluorescent dye DAPI (Courtesy of Kevin F. Sullivan)

By using the method of fluorescent microcopy, one can see in a living cell the localization (and in some cases to calculate the amount) of certain substances. It is also possible to inject fluorochrome-labeled antibodies into living cells, and to observe organized microtubules in the mitotic spindle of an animal cell (**fig. 1.5**).

An **electron microscope** is a scientific instrument which uses a beam of electrons to obtain an image of objects with a maximum magnification of up to 10^6 times. To obtain an image in an electron microscope, special magnetic lenses are used to control the movement of electrons in the column of the instrument using magnetic field (**fig. 1.6**).

In 1932, M. Knoll and E. Ruska built the first prototype of a modern electron microscope. This work was awarded the Nobel Prize in Physics in 1986. Nowadays, transmission electron microscopes and scanning (raster) electron microscopes are widely used. A transmission electron microscope uses a high-energy electron beam to form an image; however, it only gives a planar image of a micro object. To get three-dimensional images of a sample surface, scanning electron microscopes are applied.

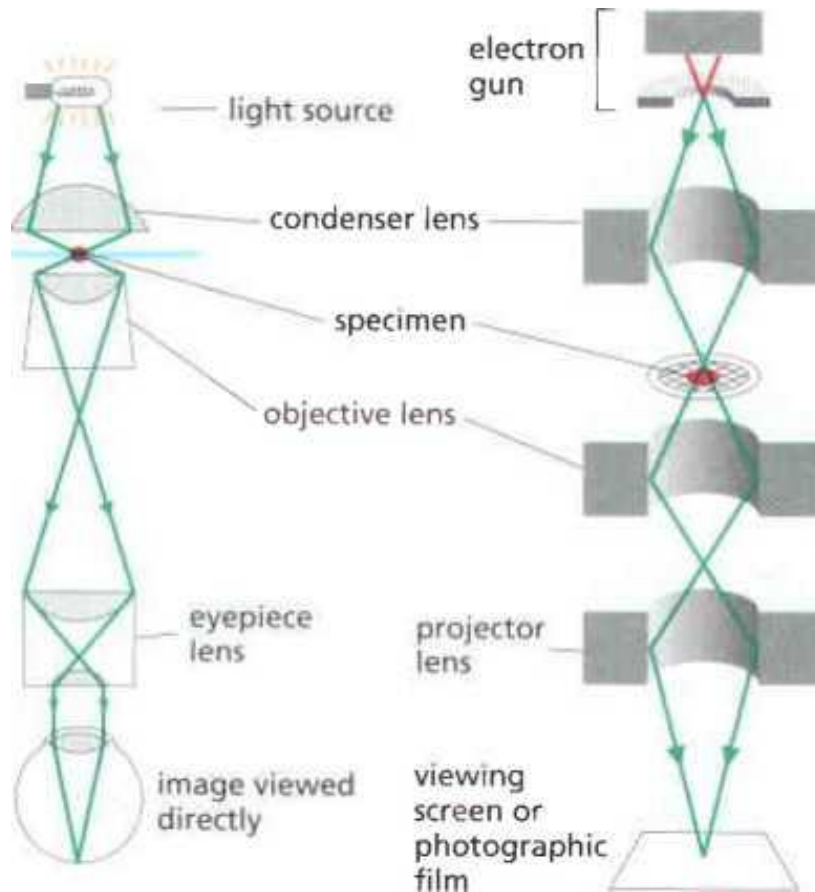


Figure 1.6. The principal features of a light microscope and a transmission electron microscope. These drawings emphasize the similarities of overall design. Whereas the lenses in the light microscope are made of glass, those in the electron microscope are magnetic coils. The electron microscope requires that the specimen be placed in a vacuum. The inset shows a transmission electron microscope in use (Alberts et al., 2008, p. 613)

They operate on the principle of scanning the object under investigation by the electron microprobe, i.e. they sequentially "touch" some points of the surface with a sharply focused electron beam, which opens up great opportunities for the application of electron microscopy literally in all fields of biology and medicine.

These techniques are only a small part of a rich arsenal of methods used in cytology to study the diversity of cellular life forms.

The series of images in **figure 1.7.** illustrate an imaginary progression from a thumb to a cluster of atoms. Each successive image represents a tenfold increase in magnification. The naked eye could see features in the first two panels, the resolution of the light microscope would extend to about the fourth panel, and the electron microscope to between about the seventh and eighth panel. **Figure 1.8.** shows the sizes of various cellular and subcellular structures and the ranges of size that different types of microscopes can visualize.

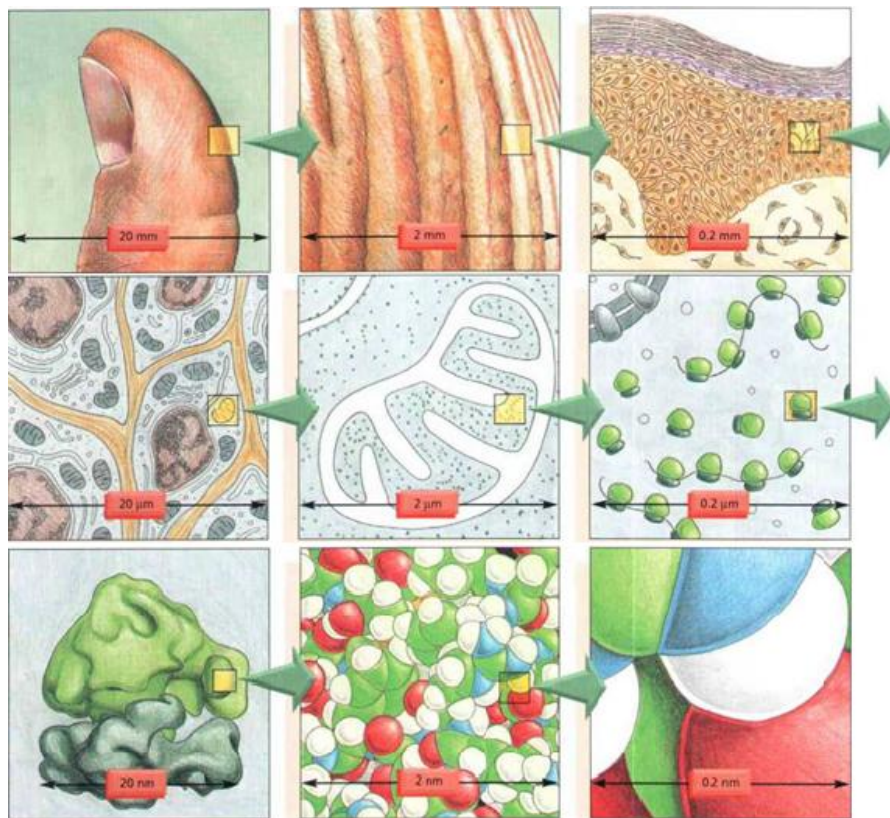


Figure 1.7. A sense of scale between living cells and atoms (Alberts et al., 2008, p. 580)

Each diagram shows an image magnified by a factor of ten in an imaginary progression from a thumb, through skin cells, to a ribosome, to a cluster of atoms forming part of one of the many protein molecules in our body. Atomic details of macromolecules, as shown in the last two panels, are usually beyond the power of the electron microscope

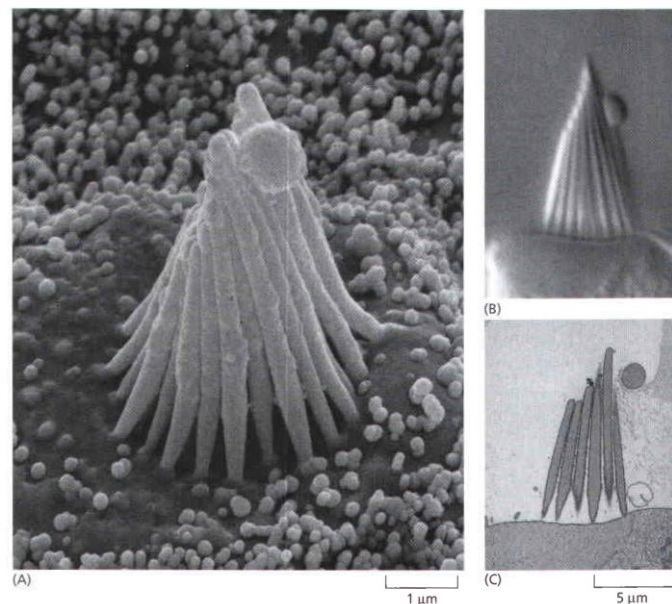


Figure 1.8. Scanning electron microscopy (Alberts B. et al., 2008, p. 643)

(A) A scanning electron micrograph of the stereocilia projecting from a hair cell in the inner ear of a bullfrog. For comparison, the same structure is shown by (B) differential-interference-contrast light microscopy and (C) thin-section transmission electron microscopy. (Courtesy of Richard Jacobs and James Hudspeth) (https://michaeldmann.net/pix_4b/hair_cell_em.gif)

Determining the detailed structure of the membranes and organelles in cells requires the higher resolution attainable in a transmission electron microscope. Specific macromolecules can be localized with colloidal gold linked to antibodies. Three-dimensional views of the surfaces of cells and tissues are obtained by scanning electron microscopy. The shapes of isolated macromolecules that have been shadowed with a heavy metal or outlined by negative staining can also be readily determined by electron microscopy. Using computational methods, either multiple images or views from different directions can be combined to produce detailed reconstructions of macromolecules and molecular complexes through the techniques of electron tomography and single-particle reconstruction, often applied to cryo-preserved specimens. The resolution obtained with these methods means that atomic structures of individual macromolecules can often be «fitted» to the images derived by electron microscopy, and that the TEM is increasingly able to completely bridge the gap between structures determined by x-ray crystallography and those determined in the light microscope.

1.3. Types of cell organization

The mechanism of biological evolution is natural selection. And what about the mechanism of pre-biological evolution? It was chemical selection. The first living pre-nuclear organisms that appeared presumably 4.0 billion years ago were anaerobic heterotrophs, i.e. they existed in an oxygen-free environment and used organic carbon sources. They had no intracellular structures and were similar to modern prokaryotes.

These organisms received food and energy from organic substances of abiogenic origin. During chemical evolution, which lasted about 1.0 billion years, conditions on the Earth changed. The stocks of organic substances, which were synthesized at early stages of evolution, were gradually exhausted, and there was a fierce competition between the first heterotrophs, which accelerated the emergence of autotrophs, organisms that use inorganic carbon sources. The very first autotrophs were capable of photosynthesis, i.e. they used solar radiation as an energy source, however, they did not produce oxygen in the process. The emergence of cyanobacteria (blue-green algae), capable of photosynthesis with the release of oxygen, resulted in oxygen accumulation in the atmosphere, which in turn contributed to the formation of the ozone layer, which protected primary organisms from hard ultraviolet radiation with a wavelength of 0.29 μm . However, the abiogenic synthesis of organic substances has stopped. In the presence of oxygen, aerobic organisms emerged.

The second most significant event of biological evolution (after the origin of life itself) is the emergence of eukaryotic cells (approximately 2.0 billion years ago). The most important difference between eukaryotic organisms and prokaryotic ones is a more perfect system of genome regulation. In eukaryotic organisms that have a separate cell nucleus, the area of active metabolism,

cytoplasm, is separated from the area where genetic information is stored, read, and replicated and, most importantly, where regulation of transcription and posttranscriptional modifications of RNA occurs.

The theory of the symbiogenetic origin of eukaryotes has now been proven and accepted. Eukaryotes are the result of symbiosis of several kinds of prokaryotes (bacteria). A heterotrophic amoeboid cell provided the main base for such symbiosis. Its food consisted of smaller cells. One of the food components of such a cell were alpha-proteobacteria (aerobic eubacteria) capable of functioning inside the host cell and synthesizing energy.

Later on, these symbiote bacteria were transformed into mitochondria. When another group of symbiotes, flagellate bacteria similar to modern spirochaetes, attached to the amoeboid host cell, flagella and cilia appeared on these cells. This type of a heterotrophic animal cell had evolutionary advantages since it could move to get its food.

Symbiosis of a heterotrophic amoeboid cell with photosynthetic cyanobacteria, blue-green algae, resulted in the formation of plastids (chloroplasts) and plant cells (**fig. 1.9**). The eukaryote genome is originally of a chimeric nature, as it is a combination of the genomes of several unrelated prokaryotes, which were combined into a single organism about 2 billion years ago.

Other compartments in eukaryotic cells may have originated in a conceptually similar way. The invagination and pinching off of specialized intracellular membrane structures from the plasma membrane creates organelles with an interior that is topologically equivalent to the exterior of the cell. We shall see that this topological relationship holds for all of the organelles involved in the secretory and endocytic pathways, including the ER, Golgi apparatus, endosomes, and lysosomes. We can therefore think of all of these organelles as members of the same family.

According to the hypothetical scheme shown in **figure 1.9B**, the inner membrane of mitochondria and plastids corresponds to the original plasma membrane of the bacterium, while the lumen of these organelles evolved from the bacterial cytosol. As we might expect from such an endocytic origin, these two organelles are surrounded by a double membrane, and they remain isolated from the extensive vesicular traffic that connects the interiors of most of the other membrane-enclosed organelles to each other and to the outside of the cell.

The evolutionary scheme described above groups the intracellular compartments in eukaryotic cells into four distinct families: (1) – the nucleus and the cytosol, which communicate with each other through nuclear pore complexes and are thus topologically continuous (although functionally distinct); (2) – all organelles that function in the secretory and endocytic pathways, including the ER, Golgi apparatus, endosomes, and lysosomes, the numerous classes of transport intermediates such as transport vesicles that move between them, and possibly peroxisomes; (3) – the mitochondria; and (4) – the plastids (in plants only) (**fig. 1.10**).

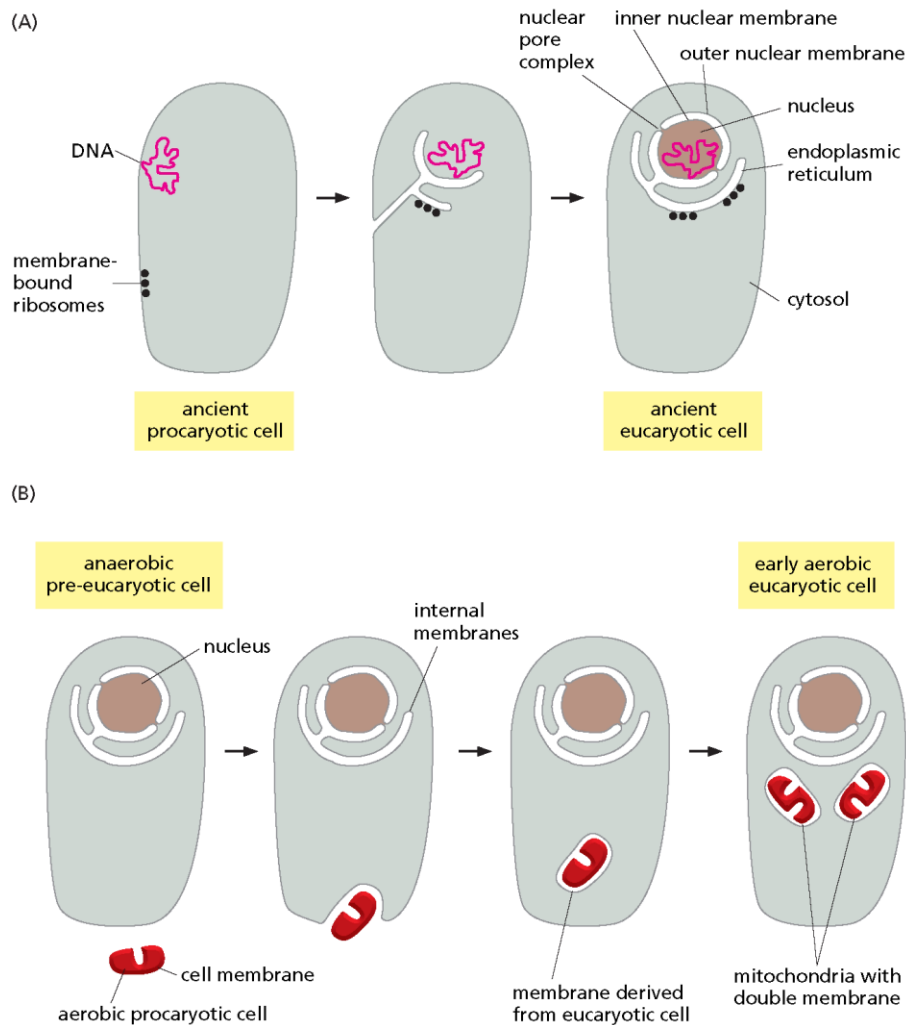


Figure 1.9. Hypothetical schemes for the evolutionary origins of some organelles (Alberts et al., 2008, p. 29)

The origins of mitochondria, chloroplasts, ER, and the cell nucleus might explain the topological relationships of these compartments in eukaryotic cells.

(A) A possible pathway for the evolution of the cell nucleus and the ER. In some bacteria the single DNA molecule that comprises the bacterial chromosome is attached to an invagination of the plasma membrane. Such an invagination in a very ancient prokaryotic cell could have rearranged to form an envelope around the DNA, while still allowing the DNA access to the cell cytosol (as is required for DNA to direct protein synthesis). This envelope is presumed to have eventually pinched off completely from the plasma membrane, producing a nuclear compartment surrounded by a double membrane. As illustrated, gated passageways called nuclear pore complexes (NPCs) penetrate the nuclear envelope. Because it is surrounded by two membranes that are in continuity where they are penetrated by NPCs, the nuclear compartment is topologically equivalent to the cytosol; in fact, during mitosis the nuclear contents mix with the cytosol. The lumen of the ER is continuous with the space between the inner and outer nuclear membranes, and it is topologically equivalent to the extracellular space.

(B) Mitochondria (and plastids) are thought to have originated when a bacterium was engulfed by a larger pre-eukaryotic cell. This could explain why they contain their own genomes and why the lumens of these organelles remain isolated from the membrane traffic that interconnects the lumens of many other intracellular compartments

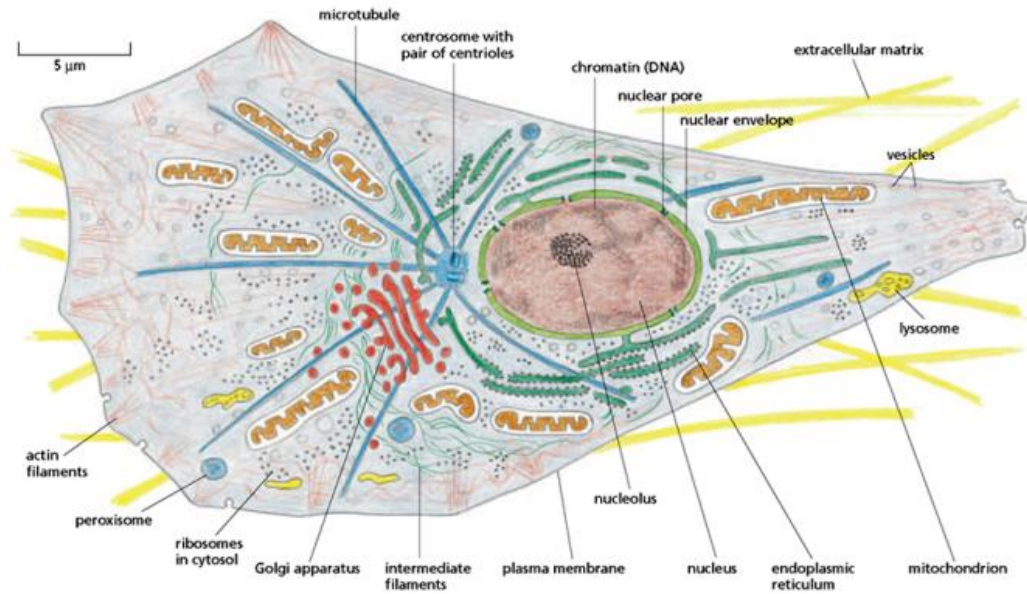


Figure 1.10. The major features of eukaryotic cells (Alberts et al., 2008, p. 27)

The drawing depicts a typical animal cell, but almost all the same components are found in plants and fungi and in single-celled eucaryotes such as yeasts and protozoa. Plant cells contain chloroplasts in addition to the components shown here, and their plasma membrane is surrounded by a tough external wall formed of cellulose

In the late 1970s, the American microbiologist Carl Woese proposed a three-domain classification of organisms. A phylogenetic tree built on the basis of analysis of rRNA gene sequences shows the common origin of organisms of all three domains: Bacteria, Archaea, and Eukaryote (**fig. 1.11**).

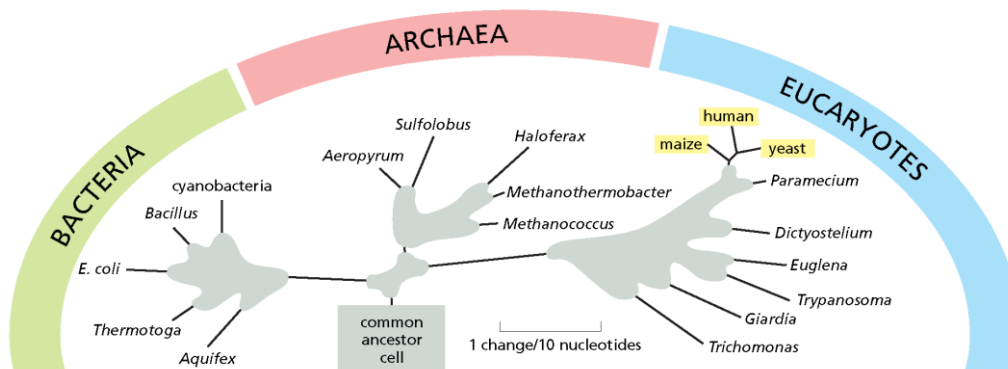


Figure 1.11. The three major divisions (domains) of the living world (Alberts et al., 2008, p. 16)

Note that traditionally the word bacteria have been used to refer to prokaryotes in general, but more recently has been redefined to refer to eubacteria specifically. The tree shown here is based on comparisons of the nucleotide sequence of a ribosomal RNA subunit in the different species, and the distances in the diagram represent estimates of the numbers of evolutionary changes that have occurred in this molecule in each lineage. The parts of the tree shrouded in gray cloud represent uncertainties about details of the true pattern of species divergence in the course of evolution: comparisons of nucleotide or amino acid sequences of molecules other than rRNA, as well as other arguments, lead to somewhat different trees. There is general agreement, however, as to the early divergence of the three most basic domains: the bacteria, the archaea, and the eucaryotes

Prokaryotes include two domains: bacteria and archaea. Archaea are older than bacteria. They show an exceptionally high resistance to extreme environmental conditions and resemble bacteria in terms of the nature of their main metabolic processes and structure. Archaea differ substantially from prokaryotes as they lack a cell wall containing peptidoglycan. Besides, the genetic apparatus of archaea is more similar to that of eukaryotes than to that of bacteria.

Archaea are often found inhabiting environments that we humans avoid, such as bogs, sewage treatment plants, ocean depths, salt brines, and hot acid springs, although they are also widespread in less extreme and more homely environments, from soils and lakes to the stomachs of cattle. In outward appearance they are not easily distinguished from bacteria. At a molecular level, archaea seem to resemble eukaryotes more closely in their machinery for handling genetic information (replication, transcription, and translation), but bacteria more closely in their apparatus for metabolism and energy conversion.

CONTROL TESTS

Read attentively each assignment (question) and choose one correct answer from the choices offered below.

1. What was the main research method used during the initial period of cytology development?

- a) experimental;
- b) observation and description of objects;
- c) modeling.

2. In which part of a light microscope is the primary image built that is geometrically similar to an object?

- a) in the eyepiece;
- b) in the objective;
- c) in the condenser.

3. Explain the term «objective lens curvature»

- a) the part of the field of view that is in focus;
- b) the ability to give a separate image of two adjacent elements;
- c) correction of chromatic aberration for two extreme wavelengths of the visible spectrum.

4. What is the resolving power of an objective?

- a) the ability to give a separate image of two adjacent elements of the specimen;
- b) the ability to enlarge the object being observed;
- c) the ability to build a primary, geometrically similar image to the object, which is enlarged with eyepiece lenses.

TOPICS FOR INDEPENDENT WORK

Independent work includes the study of the following topics:

Topic 1: Development of modern cytology. Revealing of ultramicroscopic features inherent to specialized cells.

Topic 2: Modern postulates of cell theory.

Topic 3: Modern research methods: microscopy, fractionation, cytochemical methods, radioautography, cryoultramicrotomy, immunochemistry, etc.

Topic 4: Design and principle of operation of a light microscope.

Topic 5: Hypothesis of the origin of prokaryotic cells.

Topic 6: Theory of symbiogenetic origin of eukaryotic cells.

CHEMICAL COMPOSITION OF THE CELL

There is nothing in living organisms that disobeys chemical and physical laws. However, the chemistry of life is special. First, it is based overwhelmingly on carbon compounds, whose study is therefore known as organic chemistry. Second, cells are 70 percent water, and life depends largely on chemical reactions that take place in aqueous solution. Third, and most important, cell chemistry is enormously complex: even the simplest cell is vastly more complicated in its chemistry than any other chemical system known. Although cells contain a variety of small carbon-containing molecules, most of the carbon atoms in cells are incorporated into enormous polymeric molecules, chains of chemical subunits linked end-to-end. It is the unique properties of these macromolecules that enable cells and organisms to grow and reproduce, as well as to do all the other things that are characteristic of life.

2.1. Inorganic substances

The cell consists of a large number of chemical elements of the Periodic System, with 86 elements that are constantly present, 25 that are necessary for normal vital activity of the organism, including 16–18 that are indispensable. Organogens (bioelements) are chemical elements that make part of all organic compounds and amount to about 98% of the cell mass (Table 2.1).

Table 2.1

Chemical elements (organogens) in living cells

Element	Content, %	Function
Oxygen	65–75	It is part of most organic substances in the cell. It is formed through photosynthesis during water photolysis. In aerobic organisms, it serves as an oxidizer during cell respiration by providing cells with energy. In living cells, it is mainly contained in water.
Carbon	15–18	It is part of all organic substances; their molecules are built upon a skeleton of carbon atoms. In addition, in the form of CO ₂ it is fixed in the process of photosynthesis and released during breathing. In the form of CO (in low concentrations), it is involved in the regulation of cell functions, and in the form of CaCO ₃ it makes part of the mineral skeleton.

Hydrogen	8–10	It is part of all organic substances in the cell. In living cells, it is mainly contained in water. Some bacteria oxidize molecular hydrogen to produce energy.
Nitrogen	2–3	It is part of amino acids, proteins (including enzymes and hemoglobin), nucleic acids, chlorophyll, some vitamins.

Macroelements are elements contained in the cell in relatively large amount (tenths and hundredths of a percent) (Table 2.2).

Table 2.2

Macroelements in living cells

Element	Content, %	Function
Calcium	0,04–2,00	It is found in cell membrane, intercellular matter and bones. It is involved in the regulation of intracellular processes, maintaining the membrane potential, nerve impulse transmission, it is necessary for muscle contraction and exocytosis. Soluble calcium salts participate in forming bones and teeth of vertebrates and mineral skeletons of invertebrates.
Phosphorus	0,2–1,0	It is part of ATP as a residue of phosphoric acid (PO_4^{3-}). It is found in bone tissue and tooth enamel (in the form of mineral salts) and is also present in cytoplasm and intercellular fluids (in the form of phosphate ions).
Iron	0,01%	It is contained in hemoglobin.
Iodine	0,01%	It is contained in thyroid hormones (thyroxine, triiodothyronine).
Potassium	0,15–0,4	It is involved in maintaining membrane potential, generating nerve impulses, regulating heart muscle contraction. It is contained in intercellular substances. It participates in photosynthesis.
Sulfur	0,15–0,2	It is contained in some amino acids, enzymes, thiamine. It is present in small amounts as a sulfate ion in cell cytoplasm and intercellular fluids.
Chlorine	0,05–0,1	It participates in forming the osmotic potential of blood plasma and other liquids in the form of anion.
Sodium	0,02–0,03	It participates in maintaining membrane potential, nerve impulse generation, osmoregulation processes and the creation of a blood buffer system.
Magnesium	0,02–0,03	It is a cofactor of many enzymes involved in energy exchange and DNA synthesis; it serves to maintain the integrity of ribosomes and mitochondria; it is contained in chlorophyll. In animal cells, it is essential for the functioning of the muscular and bone systems.

Microelements. The microelements that make up from 0.001% to 0.000001% of the body weight of living organisms include rubidium, cadmium, aluminum,

barium, tin, lead (necessary for optimal iron metabolism), vanadium, germanium, cobalt (vitamin B₁₂), manganese, nickel, ruthenium, selenium, fluorine (tooth enamel), copper, chromium, zinc, molybdenum (involved in the binding of atmospheric nitrogen), boron (affects germination processes in plants), as well as silicon and strontium.

Ultra-microelements account for less than 0.000001% of the weight of living organisms. They include gold and silver, which have a bactericidal effect, mercury that inhibits the back absorption of water in the kidney tubules, thus affecting the enzymes. Ultra-microelements also include selenium, arsenic, platinum and cesium, beryllium, radium, uranium, palladium, niobium, xenon, argon, krypton, helium, neon, polonium, radon, actinium, thallium, thorium, iridium, and some others. The functions of the ultra-microelements are not yet quite understood.

Water accounts for about 70% of a cell's weight, and most intracellular reactions occur in an aqueous environment. Life on Earth began in the ocean, and the conditions in that primeval environment put a permanent stamp on the chemistry of living things. Life therefore hinges on the properties of water.

In each water molecule (H₂O) the two H atoms are linked to the O atom by covalent bonds (**fig. 2.1**).

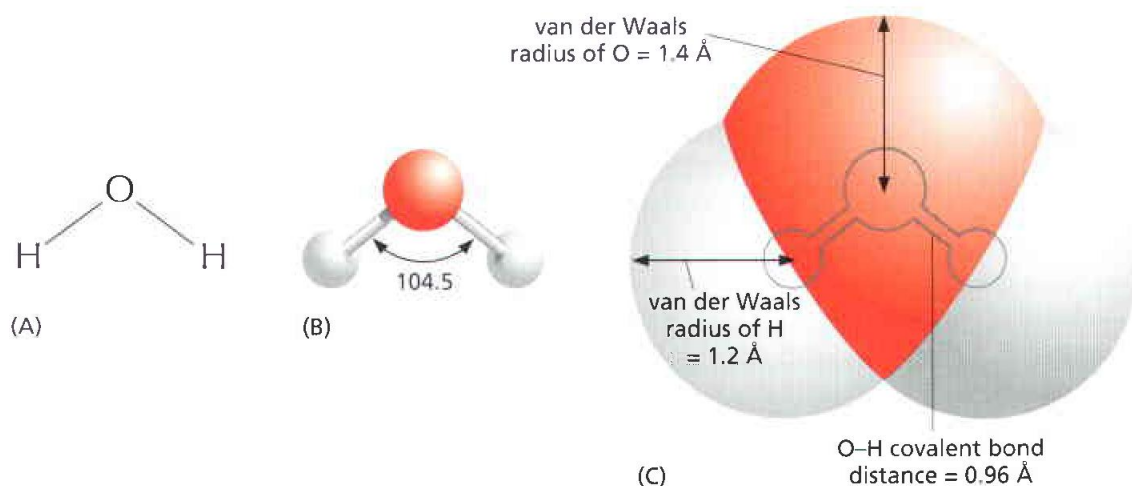


Figure 2.1. Three representations of a water molecule (Alberts et al., 2008, p. 52)

(A) The usual line drawing of the structural formula, in which each atom is indicated by its standard symbol, and each line represents a covalent bond joining two atoms. (B) A ball-and-stick model, in which atoms are represented by spheres of arbitrary diameter, connected by sticks representing covalent bonds. Unlike (A), bond angles are accurately represented in this type of model. (C) A space-filling model, in which both bond geometry and van der Waals radii are accurately represented

The two bonds are highly polar because the O is strongly attractive for electrons, whereas the H is only weakly attractive. Consequently, there is an unequal distribution of electrons in a water molecule, with a preponderance of positive charge on the two H atoms and of negative charge on the O. When a positively charged region of one water molecule (that is, one of its H atoms)

approaches a negatively charged region (that is, the O) of a second water molecule, the electrical attraction between them can result in a weak bond called a hydrogen bond. These bonds are much weaker than covalent bonds and are easily broken by the random thermal motions due to the heat energy of the molecules, so each bond lasts only a short time. But the combined effect of many weak bonds can be profound. Each water molecule can form hydrogen bonds through its two H atoms to two other water molecules, producing a network in which hydrogen bonds are being continually broken and formed. It is only because of the hydrogen bonds that link water molecules together that water is a liquid at room temperature, with a high boiling point and high surface tension, rather than a gas.

Molecules, such as alcohols, that contain polar bonds and that can form hydrogen bonds with water dissolve readily in water. Molecules carrying plus or minus charges (ions) likewise interact favorably with water. Such molecules are termed hydrophilic, meaning that they are water-loving. A large proportion of the molecules in the aqueous environment of a cell necessarily fall into this category, including sugars, DNA, RNA, and most proteins. Hydrophobic (water hating) molecules, by contrast, are uncharged and form few or no hydrogen bonds, and so do not dissolve in water. Hydrocarbons are an important example. In these molecules the H atoms are covalently linked to C atoms by a largely nonpolar bond. Because the H atoms have almost no net positive charge, they cannot form effective hydrogen bonds to other molecules.

2.2. Organic compounds

Carbon atoms can form chains and rings and hence generate large and complex molecules with no obvious upper limit to their size. The small and large carbon compounds made by cells are called organic molecules. Certain combinations of atoms, such as the methyl ($-\text{CH}_3$), hydroxyl ($-\text{OH}$), carboxyl ($-\text{COOH}$), carbonyl ($-\text{C}=\text{O}$), phosphate ($-\text{PO}_3^{2-}$), sulfhydryl ($-\text{SH}$), and amino ($-\text{NH}_2$) groups, occur repeatedly in organic molecules. Each such chemical group has distinct chemical and physical properties that influence the behavior of the molecule in which the group occurs.

The small organic molecules of the cell are carbon-based compounds that have molecular weights in the range 100-1000 and contain up to 30 or so carbon atoms. They are usually found free in solution and have many different fates. Some are used as monomer subunits to construct the giant polymeric macromolecules – the proteins, nucleic acids, and large polysaccharides – of the cell. Others act as energy sources and are broken down and transformed into other small molecules in a maze of intracellular metabolic pathways. Many small molecules have more than one role in the cell, for example, acting both as a potential subunit for a macromolecule and as an energy source. Small organic molecules are much less abundant than the organic macromolecules, accounting for only about one-tenth of the total mass of

organic matter in a cell (Table 2.3). As a rough guess, there may be a thousand different kinds of these small molecules in a typical cell.

Table 2.3

The Types of Molecules That Form a Bacterial Cell

(Alberts et al., 2008, p. 55)

	Percent of total cell weight	Number of types of each molecule
Water	70	1
Inorganic ions	1	20
Sugars and precursors	1	250
Amino acids and precursors	0.4	100
Nucleotides and precursors	0.4	100
Fatty acids and precursors	1	50
Other small molecules	0.2	≈300
Macromolecules (proteins, nucleic acids, and polysaccharides)	26	≈3000

Cells contain four major families of small organic molecules: the sugars, the fatty acids, the amino acids, and the nucleotides (*fig. 2. 2*).

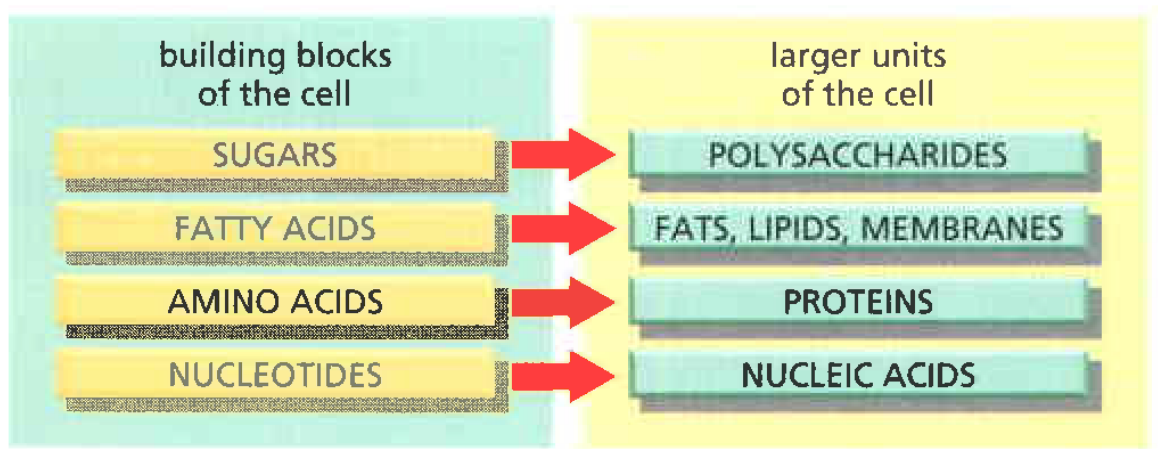


Figure 2.2. The four main families of small organic molecules in cells (Alberts et al., 2008, p. 56) These small molecules form the monomeric building blocks, or subunits, for most of the macromolecules and other assemblies of the cell. Some, such as the sugars and the fatty acids, are also energy sources

A nucleotide is a molecule made up of a nitrogen-containing ring compound linked to a five-carbon sugar, which in turn carries one or more phosphate groups. The five-carbon sugar can be either ribose or deoxyribose. Nucleotides containing ribose are known as ribonucleotides, and those containing deoxyribose as deoxyribonucleotides. The nitrogen-containing rings are generally referred to as

bases for historical reasons: under acidic conditions they can each bind an H^+ (proton) and thereby increase the concentration of OH^- ions in aqueous solution. There is a strong family resemblance between the different bases. Cytosine (C), thymine (T), and uracil (U) are called pyrimidines because they all derive from a six-membered pyrimidine ring; guanine (G) and adenine (A) are purine compounds, and they have a second, five-membered ring fused to the six-membered ring. Each nucleotide is named for the base it contains.

Nucleotides can act as short-term carriers of chemical energy. Above all others, the ribonucleotide adenosine triphosphate, or ATP (**fig. 2.3**), transfers energy in hundreds of different cell reactions. ATP is formed through reactions that are driven by the energy released by the oxidative breakdown of foodstuffs. Its three phosphates are linked in series by two phosphoanhydride bonds, whose rupture releases large amounts of useful energy. The terminal phosphate group in particular is frequently split off by hydrolysis, often transferring a phosphate to other molecules and releasing energy that drives energy-requiring biosynthetic reactions. Other nucleotide derivatives are carriers for the transfer of other chemical groups, as will be described later.

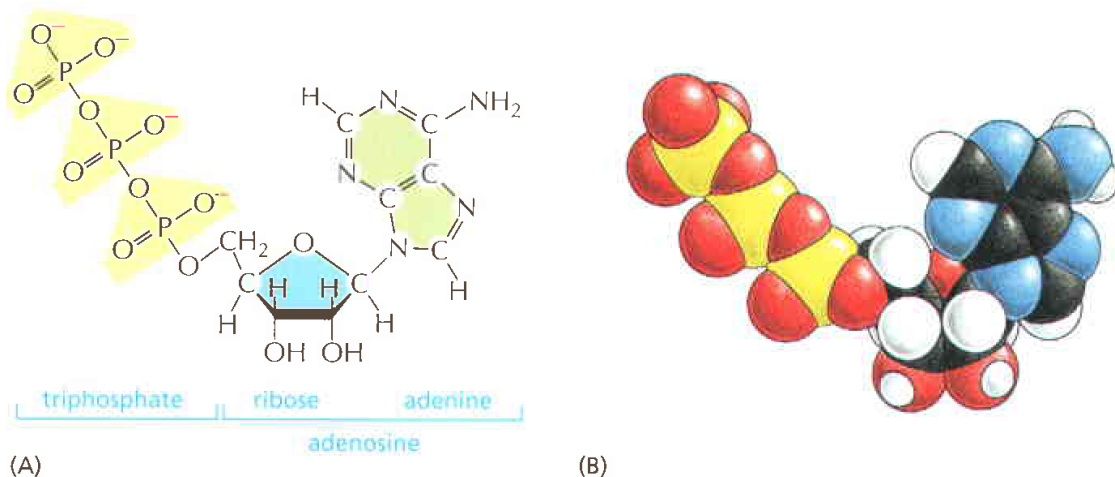


Figure 2.3. Chemical structure of adenosine triphosphate (ATP) (Alberts et al., 2008, p. 61)

(A) Structural formula. (B) Space-filling model. In (B) the colors of the atoms are C, black; N, blue; H, white; O, red; and P, yellow

The most fundamental role of nucleotides in the cell, however, is in the storage and retrieval of biological information. Nucleotides serve as building blocks for the construction of nucleic acids—long polymers in which nucleotide subunits are covalently linked by the formation of a phosphodiester bond between the phosphate group attached to the sugar of one nucleotide and a hydroxyl group on the sugar of the next nucleotide (**fig. 2.4**). Nucleic acid chains are synthesized from energy-rich nucleoside triphosphates by a condensation reaction that releases inorganic pyrophosphate during phosphodiester bond formation.

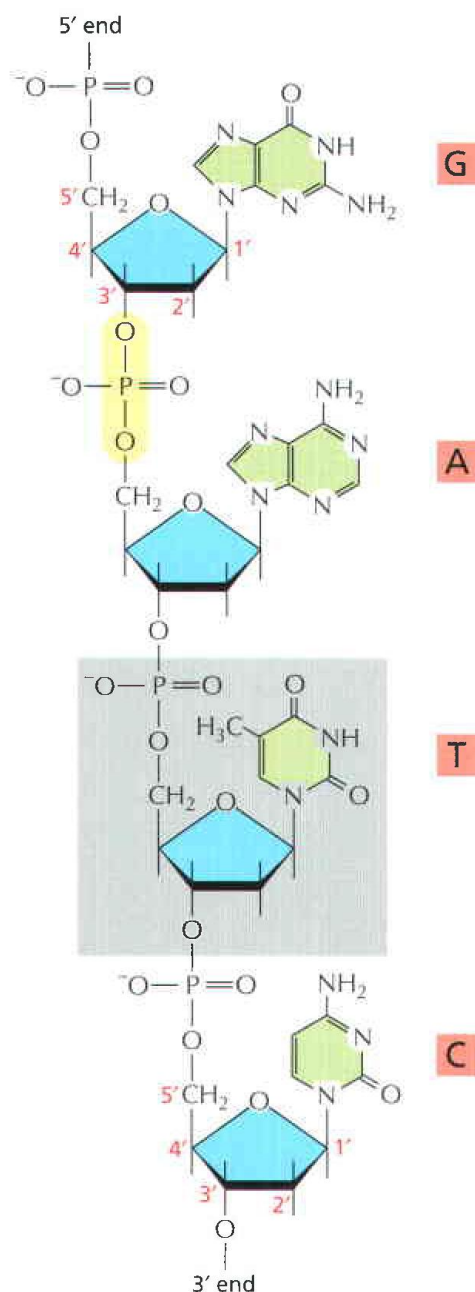


Figure 2.4. A small part of one chain of a deoxyribonucleic acid (DNA) molecule (Alberts et al., 2008, p. 62)

Four nucleotides are shown. One of the phosphodiester bonds that links adjacent nucleotide residues is highlighted in yellow, and one of the nucleotides is shaded in gray. Nucleotides are linked together by a phosphodiester linkage between specific carbon atoms of the ribose, known as the 5' and 3' atoms. For this reason, one end of a polynucleotide chain, the 5' end, will have a free phosphate group and the other, the 3' end, a free hydroxyl group. The linear sequence of nucleotides in a polynucleotide chain is commonly abbreviated by a one-letter code, and the sequence is always read from the 5' end. In the example illustrated the sequence is G-A-T-C

There are two main types of nucleic acids, differing in the type of sugar in their sugar-phosphate backbone. Those based on the sugar ribose are known as ribonucleic acids, or RNA, and normally contain the bases A, G, C, and U. Those

based on deoxyribose (in which the hydroxyl at the 2' position of the ribose carbon ring is replaced by a hydrogen are known as deoxyribonucleic acids, or DNA, and contain the bases A, G, C, and T (T is chemically similar to the U in RNA, merely adding the methyl group on the pyrimidine ring). RNA usually occurs in cells as a single polynucleotide chain, but DNA is virtually always a double-stranded molecule, a DNA double helix composed of two polynucleotide chains running antiparallel to each other and held together by hydrogen-bonding between the bases of the two chains (*fig. 2.5*).

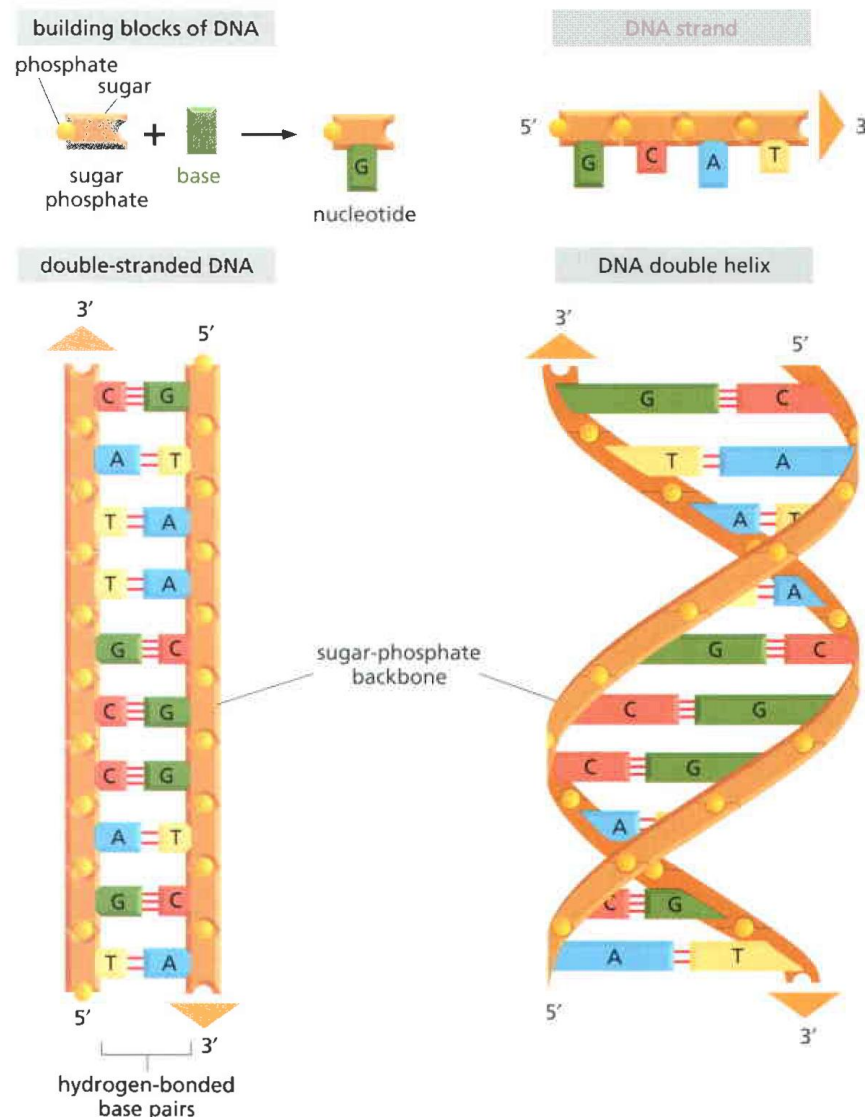


Figure 2.5. DNA and its building blocks (Alberts et al., 2008, p. 198)

DNA is made of four types of nucleotides, which are linked covalently into a polynucleotide chain (a DNA strand) with a sugar-phosphate backbone from which the bases (A, C, G, and T) extend. A DNA molecule is composed of two DNA strands held together by hydrogen bonds between the paired bases. The arrowheads at the ends of the DNA strands indicate the polarities of the two strands, which run antiparallel to each other in the DNA molecule. In the diagram at the bottom left of the figure, the DNA molecule is shown straightened out; in reality, it is twisted into a double helix, as shown on the right

The linear sequence of nucleotides in a DNA or an RNA encodes the genetic information of the cell. The ability of the bases in different nucleic acid molecules to recognize and pair with each other by hydrogen-bonding (called base-pairing): G with C, and A with either T or U, underlies all of heredity and evolution.

CONTROL TESTS

Read attentively each assignment (question) and choose one correct answer from the choices offered below.

1. The content of which of the chemical elements is the smallest in the cells?

- a) nitrogen;
- b) oxygen;
- c) carbon;
- d) hydrogen.

2. Which of the properties of water is due to its polarity?

- a) heat conductivity;
- b) heat capacity;
- c) the ability to dissolve non-polar compounds;
- d) the ability to dissolve polar compounds.

3. What are the chemical bonds between atoms in the water molecule?

- a) non-polar covalent;
- b) polar covalent;
- c) ionic;
- d) hydrogen.

4. The greatest amount of energy is released with the breakdown of one gram of

- a) fat;
- b) glucose;
- c) protein.

TOPICS FOR INDEPENDENT WORK

Independent work includes the study of the following topics:

Topic 1: Atomic (elemental) composition of the cell, organogens, macroelements, microelements, ultra-microelements.

Chapter 2: Chemical composition of the cell

Topic 2: Molecular composition of the cell. Systematize and give examples of inorganic and organic substances in the cell.

Topic 3: Properties of water and inorganic ions and their role in the cell (organism).

Topic 4: Main groups of organic compounds in the cell, their role in the cell (organism).

PROKARYOTIC CELL BIOLOGY

3.1. Features of organization and diversity of prokaryotes

Prokaryotes or «pre-nuclear» organisms are incredibly diverse in their metabolic processes and occupy a wide variety of ecological niches. They can exist in the harshest environmental conditions: at temperatures above 100°C, under high and low pressure. Their growth is influenced by such factors as the acidity of the environment, the presence of oxygen and water, the value of osmotic pressure and others. Depending on their shape, bacteria are classified into: spherical (cocci), rod (bacilli), spiral (spirilla), comma (vibrios) or corkscrew (spirochaetes) and some others. Some prokaryotes enter into complex symbiotic interactions, for example, root nodule bacteria synthesize ammonia from atmospheric nitrogen, which is used by plants. At the pinnacle of prokaryotes' organisation are biofilms, communities of many species of microorganisms. Individual organisms in the biofilm are embedded in a polysaccharide matrix, which in this case performs a protective function. Biofilm formation includes the stage of adhesion of bacteria on the surface and the stage of formation of colonies, which are bonded to each other through extracellular polysaccharides.

Prokaryotes are highly organized cells, they are simpler compared to eukaryotes, but their structure is not primitive. They have no membrane-enclosed nucleus, one ring-shaped DNA molecule forms a nucleoid or nucleoplasm. Prokaryotic DNA contains up to 5×10^6 pairs of nitrogenous bases, and is up to several mm long. For example, the human *E. coli* symbiote has a giant cyclic DNA molecule about 1.6 mm long. The smallest ringed DNA, 0.25 mm long, is found in mycoplasma cells. There are no internal membranes, but specific prokaryotic proteins are confined to certain subcellular areas.

Prokaryotic cells contain ribosomes that provide protein biosynthesis. These are smaller than in eukaryotes. Many bacteria can exist independently and are capable of locomotion due to two types of cell surface appendages. The first type is pili (or fimbriae). The pili are filamentous protein oligomers on the cell surface. The second type is flagella. The flagella of bacteria have a very complex structure and are fundamentally different from those of eukaryotic cells (*fig. 3.1*).

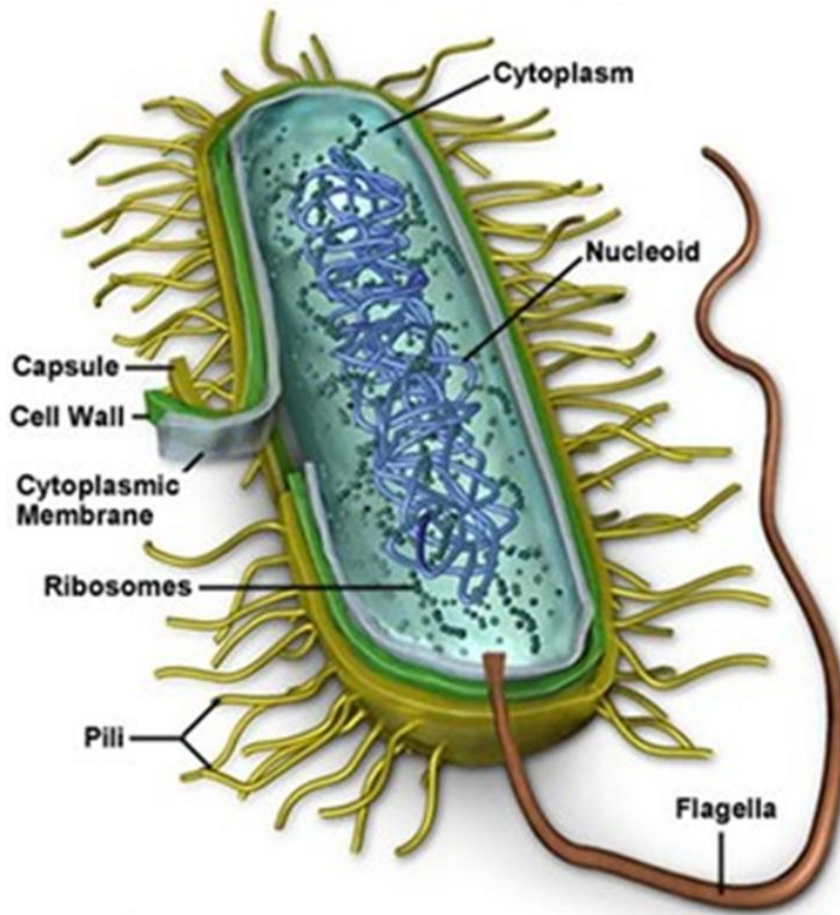


Figure 3.1. Prokaryotic cell structure (https://studopedia.ru/10_251584_morfologiya-bakteriy.html)

The giant ring DNA of prokaryotes, the nucleode (a), using RNA and proteins, is repeatedly folded to form numerous spiral loops protruding from the dense central region (b), resulting in a significant nucleoid compaction (**fig. 3.2**). One such loop, or domain, contains up to 10–15 μm of DNA. The number of loops can reach 100–120. It should be noted that part of the nucleoid DNA is associated with a small number of special basic proteins that differ from eukaryote histones. The DNA ring molecule represents one unit of replication, a replicon. The rate of replication is about 30 μm per minute. The average time between bacterial cell divisions is 20–40 minutes.

A distinctive feature of prokaryotes' nuclear structures is that RNA synthesis and protein synthesis can occur in them simultaneously: ribosomes bind to the not yet fully synthesized molecules of mRNA and perform protein synthesis on them. Thus, a triple synthetic complex emerges: DNA – the synthesizing RNA chain – ribosomes with the synthesized polypeptide chain. Consequently, transcription and translation processes are not separated territorially in prokaryotes (**fig. 3.3**).

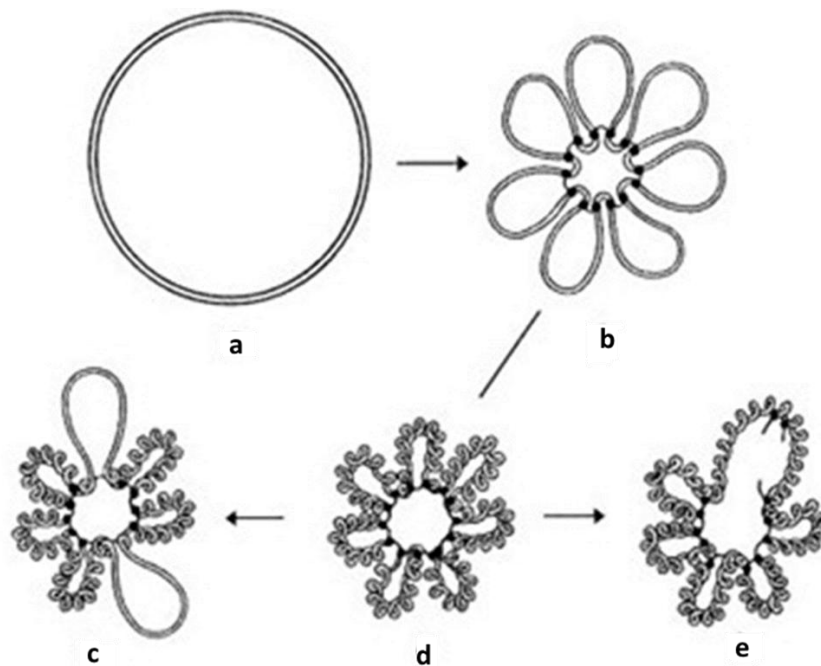


Figure 3.2. Model of bacterial DNA condensation (Chentsov, 2015, p. 64)

a – ring chromosome; b – protein links form loop domains; c – superspiralization of domains; d and e – are various forms of nucleoid decondensation

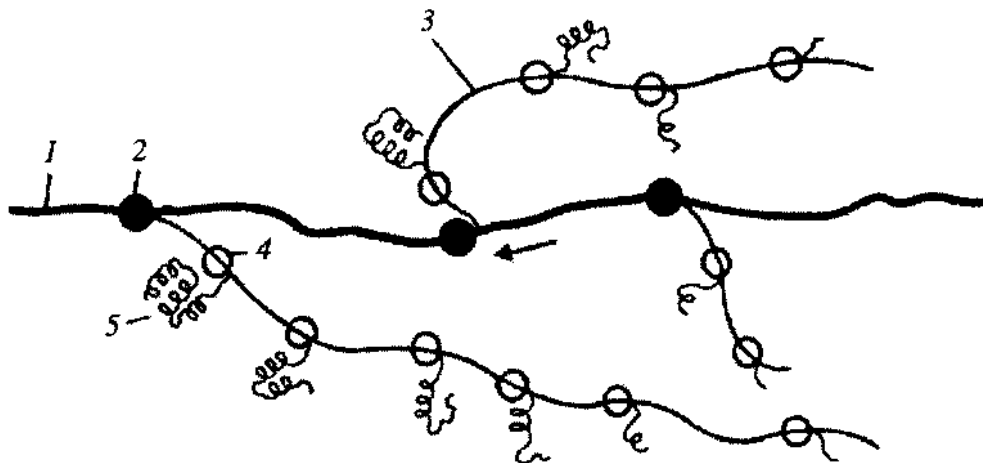


Figure 3.3. DNA and RNA synthesis in prokaryotic cells occurs simultaneously (Chentsov, 2015, p. 66)

1 – DNA; 2 – RNA polymerase; 3 – mRNA; 4 – ribosome; 5 – polypeptide

3.2. Features of prokaryote cell cycle and surface apparatus

The surface apparatus of prokaryotes includes several structures: cytoplasmic membrane, cell wall and capsule (or glycocalyx). The bacterial cell wall can account for up to 20–30% of the bacteria dry weight. Its main component is peptidoglycan or murein. The rigid framework surrounding the bacterial cell is a single giant, bag-shaped molecule of a complex polysaccharide peptide. This skeleton is known as the murein sacculus. It is made up of a network of parallel polysaccharide chains built of alternating saccharides, crosslinked by numerous

peptide transverse bonds. The length of glycan chains can reach several hundred disaccharide blocks. The basis of the peptide part of the murein are tetrapeptides formed by various amino acids. In addition to the multilayer murein skeleton, the cell wall contains a large number of additional components: teichoic acids, polysaccharides, polypeptides and proteins (**fig. 3.4**).

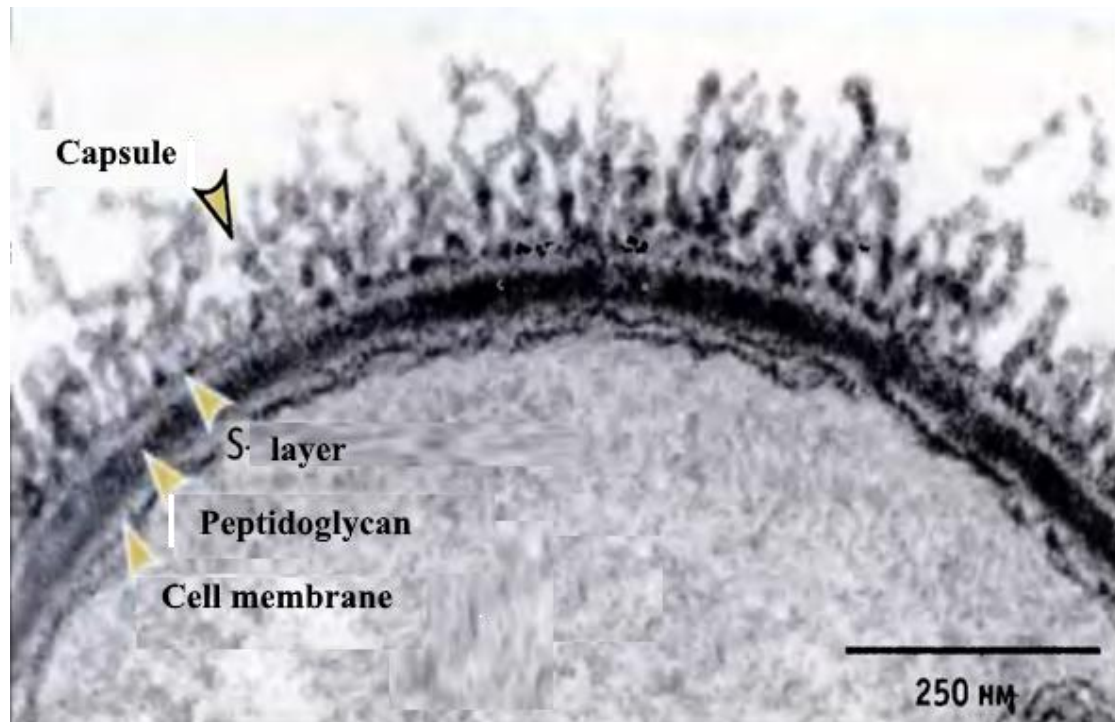


Figure 3.4. Surface apparatus (envelope) of prokaryotes (Cells, 2011, p. 810)

The photo was obtained by the authors: Mesnage S., Tosi-Couture E., Gounon P., Mock M, Fouet A. J. Bacteriol., 1998, vol. 180, p. 52–58

Depending on the structure of their envelope, bacteria are divided into Gram (+) and Gram (-). Gram (+) bacteria retain the colour of the crystal violet *stain* in the Gram stain test after iodine treatment and washing with alcohol. Gram (-) bacteria are bacteria that do not retain the crystal violet stain used in the Gram staining method of bacterial differentiation.

Gram-positive bacteria have a cell wall that is very stiff, its murein network is multi-layered, and there is a single-layer cell membrane.

Gram-negative bacteria are also characterized by the presence of an outer membrane. The cell walls of Gram-negative bacteria contain a single-layer murein network, which constitutes 12% of the dry weight of the wall. Other components account for up to 80% of the dry weight. These are lipoproteins, complex lipopolysaccharides. They form a complex outer lipoprotein membrane. Thus, the periphery of Gram-negative bacteria contains an outer membrane, then a single-layer murein network, below it there is a plasma membrane. The outer membrane ensures structural integrity of the cell and serves as a barrier to the free access of substances to the plasma membrane. It contains porins, proteins that form pores

with different permeability. Pores are involved in the transport of many low-molecular substances (sugars, amino acids, small oligosaccharides and peptides).

In prokaryotes, the process of cell body division (cytotomy) is not related to the end of DNA synthesis, because the second or even third cycle of DNA replication may begin before the cell division. This continuous DNA synthesis in fast-growing cultures results in one ring chromosome for each divided cell at the intermediate stages of its further doubling, that is why daughter cells immediately after the division already contain partially replicated genome. Several groups of special proteins take part in the nucleoid divergence process.

At the final stage of division, a constriction or septum (for example, in Gram-negative bacteria) or a partition (in Gram-positive bacteria) is formed dividing the mother cell into two daughter cells (*fig. 3.5*).

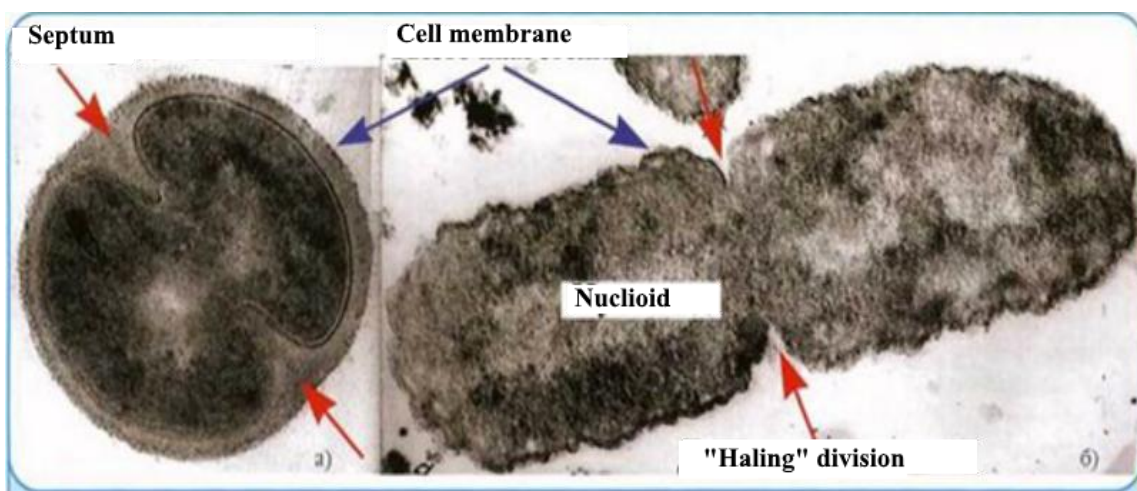


Figure 3.5. Prokaryote cytotomy (<https://studfile.net/preview/8206354/page:4/>)

The formation of a constriction, or septum, also resembles, in general terms, the cytotomy of animal cells. In this case, the formation of septum involves fibrillary thermosensitive proteins. This is a group of several proteins, the most extensively researched example being protein Z. When interacting with GTP in vitro, it can form long protofilaments. During cell division, all this protein is localized in the septum zone where it forms a contractile ring. In parallel to the formation of the septum, a buildup occurs of the murein layer of the bacterial cell wall due to the multienzyme complex that synthesizes peptidoglycans.

Thus, the division of bacterial cells involves some processes that in many ways are similar to the division of eukaryotes. Firstly, it is the divergence of chromosomes (nucleoids) due to the interaction of motor and fibrillar proteins, and secondly, the formation of a constriction due to fibrillar proteins that form a contractile ring. It is important to keep in mind that in bacteria, unlike eukaryotes, quite different proteins take part in these processes, but the principles of organization of separate stages of cell division are very similar. This type of division is called «binary fission» and results in a very accurate distribution of genetic material, almost without any errors.

CONTROL TESTS

Read attentively each assignment (question) and choose one correct answer from the choices offered below:

1. Give an explanation of the term «thermophiles»:

- a) bacteria whose intensive growth is observed at high temperatures above 50°C;
- b) bacteria whose intensive growth is observed at low temperatures, below 50°C;
- c) bacteria whose intensive growth is observed at high humidity.

2. A biofilm is

- a) a microbial community;
- b) a surface membrane of gram-negative bacteria;
- c) an outer surface of the plasmalemma.

3. Prokaryotes contain one of the following organoids:

- a) nucleus;
- b) plastids;
- c) mitochondria;
- d) ribosomes with a sedimentation constant of 80 S;
- e) ribosomes with a sedimentation constant of 70 S.

4. Explain the term «nucleoid».

- a) a ring molecule of DNA in prokaryotic cells;
- b) a linear DNA molecule in prokaryotic cells;
- c) an inner membrane of prokaryotic cells.

TOPICS FOR INDEPENDENT WORK

Independent work includes the study of the following topics:

Topic 1: Compare the structure and metabolism of different types of bacteria.

Topic 2: Study the structure of prokaryotic cell appendages: pili (or fimbriae).

Topic 3: Study the structure of prokaryotic cell appendages: flagella. What is the principle of motion of bacterial flagella?

Topic 4: Characterize the similarities of prokaryotes and archaea with eukaryotic cells.

Topic 5: Describe significant differences in the structure of archaea and prokaryotes.

Topic 6: Conduct an analysis of the role of prokaryotes in the biosphere and answer the question: Do you agree with the statement that bacteria do more harm than good?

CELL MEMBRANES

4.1. Lipid bilayer, the structural base of membranes

There are two kinds of *membranes* in the cells: *plasma membranes* and *intracellular membranes*. The surface peripheral structure that separates the interior of the cell from the outside environment is called a plasma membrane or plasmalemma. The inner membranes in the cell form cavities and thus divide the cytoplasm into a group of different compartments. The inner contents of these compartments or vacuoles is always different from the main contents of the cell, the hyaloplasm.

Biological membranes consist of a continuous double layer of lipid molecules into which membrane proteins are embedded. Depending on the type, membranes contain 25–60% lipids and 40–75% proteins. Many membranes also contain carbohydrates, their amount can reach 2–10%.

The hypothesis that the base of the cell membrane is a lipid double layer was proposed in 1925 by Dutch researchers E. Gortner and F. Grendel.

The idea that proteins attach to membranes was first expressed by J. Danielli in 1935, and in 1959 J. D. Robertson proposed the concept of unitary (or uniform) structure of all cell membranes. By the early 1970s, there were sufficiently many new facts, based on which S.J. Singer and G.L. Nicolson in 1971 proposed a modern liquid-mosaic membrane model. According to this model, the structural basis of biological membranes is a double layer of lipids (a bilayer) in which protein molecules capable of moving through the membrane are immersed or embedded (**fig. 4.1**).

Let us discuss in more detail the characteristics of lipids, the main structural components of the membrane. Lipids include a large group of organic substances that are hydrophobic (showing poor solubility in water) and lipophilic (showing good solubility in organic solvents). Typical representatives of lipids found in cell membranes are phospholipids (glycerophosphatides or glycerolipids), sphingomyelins and cholesterol from the group of steroid lipids.

Phospholipids are a major component (up to 40%) of the cell membrane. Phospholipids are esters of glycerol triatomic alcohol with two fatty acids and phosphoric acid, which in turn may be associated with different chemical groups (choline, serine, inositol, ethanolamine, etc.). A characteristic feature of lipids is that their molecules are divided into two functionally different parts: non-polar (non-charge-bearing) tails consisting of fatty acids and charged polar heads. The presence of non-polar lipid tails explains their good solubility in fats and organic solvents. Polar heads carry negative charges or may be neutral (if they have both positive and negative charges) (**fig. 4.2**).

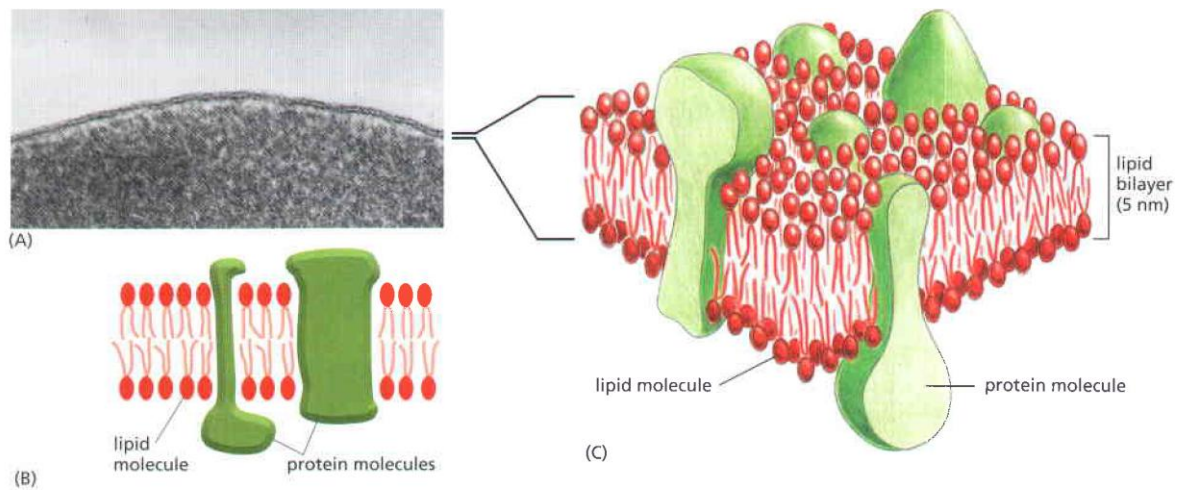


Figure 4.1. Three views of a cell membrane (Alberts et al., 2008, p. 618)

(A) An electron micrograph of a plasma membrane (of a human red blood cell) seen in cross section. (B and C) These drawings show two-dimensional and three-dimensional views of a cell membrane and the general disposition of its lipid and protein constituents

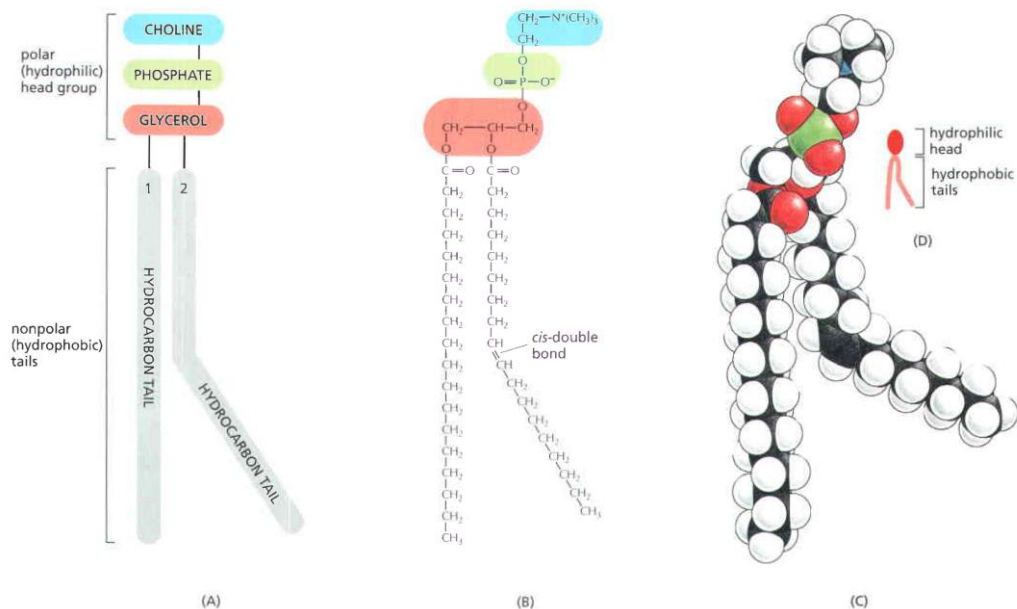


Figure 4.2. The parts of a phosphoglyceride molecule (Alberts et al., 2008, p. 619)

This example is a phosphatidylcholine, represented (A) schematically, (B) by a formula, (C) as a space-filling model, and (D) as a symbol. The kink resulting from the *c/s*-double bond is exaggerated for emphasis.

If lipid molecules are mixed with water, an emulsion consisting of micelles is formed. The micelles are the simplest aggregates formed by lipid molecules in the bulk phase of the solvent. Depending on the nature of the solvent, lipids can form either micelles of the classical type or so-called «inverted» micelles.

In classical micelles, the hydrophilic polar heads of lipid molecules are directed towards the aqueous phase, while the non-polar hydrocarbon chains form a hydrophobic nucleus isolated from the water environment. If, on the contrary, some water is added to the lipids, inverse micelles are formed: their hydrophobic tails will extend into the oil phase, while their charged (hydrophilic) heads will

group at the center of the micelle. It should be noted that in inverted micelles existing in solvents such as benzene, hexane, etc., lipid molecules have a different orientation: their hydrophobic chains extend into the solvent and polar heads form the central hydrophilic region of the micelle.

At the interface of the two aqueous phases, polar lipids easily and spontaneously form very thin bilayers. The bilayer, or double lipid layer, is thermodynamically the most advantageous form of association of those lipids whose molecules are capable of forming small micellar-type aggregates in water. As a rule, the bilayer is readily formed by lipids having little difference between the polar head area and the cross-section of hydrocarbon chains. This aspect ratio is typical of most phospholipids, which are the main components of biological membranes.

Depending on temperature, the lipid bilayer can exist in two main phase states: crystalline (or gel) and liquid crystal phase. These states are commonly referred to as «solid» and «liquid», since the physical meaning of the transition between them is the melting or freezing of the hydrocarbon chains of lipid molecules. The transition of a bilayer from crystalline to liquid crystalline state (and back) occurs at a strictly defined temperature typical for a given lipid (phase transition temperature). Lipid molecules can move along the lipid layer (lateral diffusion), can rotate around their axis (rotational diffusion), and also can move from one layer to the other (flip-flop), which occurs rarely and with the help of special carriers (**fig. 4.3**).

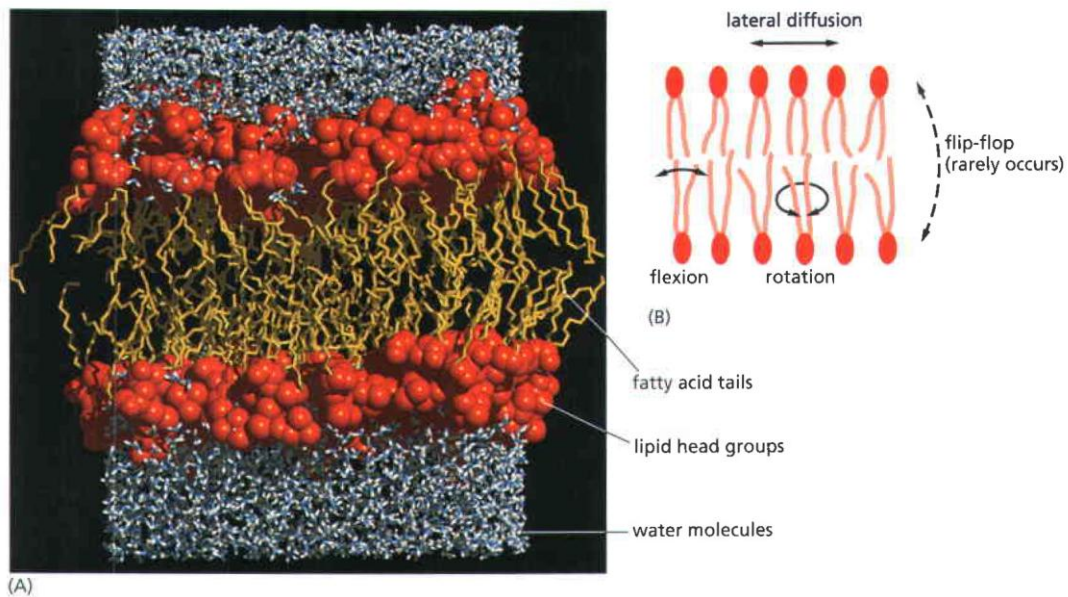


Figure 4.3. The mobility of phospholipid molecules in an artificial lipid bilayer (Alberts et al., 2008, p. 623)

Starting with a model of 100 phosphatidylcholine molecules arranged in a regular bilayer, a computer calculated the position of every atom after 300 picoseconds of simulated time. From these theoretical calculations (taking weeks of processor time in 1995), a model of the lipid bilayer emerges that accounts for almost all of the measurable properties of a synthetic lipid bilayer, such as its thickness, number of lipid molecules per membrane area, depth of water penetration, and unevenness of the two surfaces. Note that the tails in one monolayer can interact with those in the other monolayer, if the tails are long enough. (B) The different motions of a lipid molecule in a bilayer. (A, based on S.W. Chiu et al., *Biophys. J.* 69:1230–1245, 1995. With permission from the Biophysical Society)

4.2. Asymmetry of cell membranes

Despite the similarity of membrane structure, the physical and chemical properties of different membranes are different and depend on the amount and composition of lipids, proteins and carbohydrates. The qualitative and quantitative composition of lipids in the membranes is different. Thus, the plasma membrane contains 35–40% lipids, mitochondrial membranes have 27–29%, while membranes of Schwann's cells that form the myelin nerve envelope, up to 80% lipids. Plasma membranes of animal cells are rich in cholesterol (up to 30%) and contain little lecithin, while mitochondrial membranes, on the contrary, are rich in phospholipids and poor in cholesterol.

Of the total lipid content, phosphatidylcholine (lecithin) in the endoplasmic network fractions amounts to 60–70% of all phospholipids, while in the plasma membrane its content can be 25–35%. In general, the plasmalemma has a high content of cholesterol and sphingolipids, with the predominance of saturated and monounsaturated fatty acids in the phospholipids, while mitochondria, endoplasmic network and many other intracellular membranes contain little cholesterol and sphingolipids and have a relatively high content of polyunsaturated fatty acids. As a result, cytoplasmic membranes are less rigid than the plasma membrane.

Cell membranes are asymmetric in terms of their qualitative and quantitative composition of lipids and proteins (**fig. 4.4**). It has been shown that the outer surface of the plasma membrane contains approximately 80% of sphingo-myelin and 75% of the total content of phosphatidylcholine (lecithin), while the inner surface contains the whole amount of phosphatidylserine and 80% of phosphatidylethanolamine.

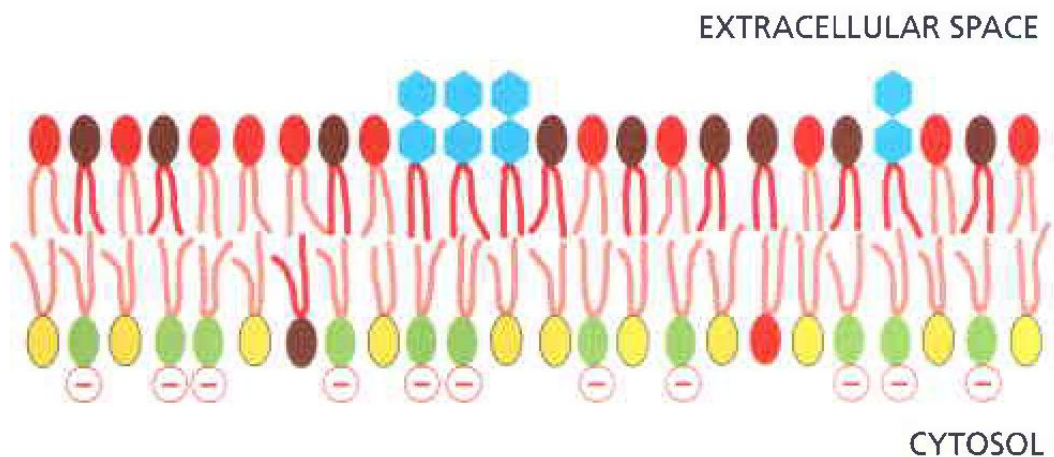


Figure 4.4. The asymmetrical distribution of phospholipids and glycolipids in the lipid bilayer of human red blood cells (Alberts et al., 2008, p. 626)

The colors used for the phospholipid head groups: *yellow* – phosphatidylethanolamine; *green* – phosphatidylserine; *red* – phosphatidylcholine; *brown* – sphingomyelin. In addition, glycolipids are drawn with hexagonal polar head groups (*blue*). Cholesterol (not shown) is thought to be distributed roughly equally in both monolayers

4.3. Membrane proteins

Membrane proteins are embedded into the lipid bilayer. On average, membranes contain 50% proteins by weight, but the amount of proteins in different membranes may vary. Thus, in mitochondrial membranes, proteins account for about 75%, while in the plasma membrane of the cells of the nerve myelin sheath, about 25%. However, since lipid molecules have a small size (about 0.5 nm) and a low molecular weight, their number in relation to the number of protein molecules is 50 times higher. Therefore, protein molecules appear to be interspersed throughout the lipid bilayer of the membrane. Some of them are linked to lipid heads by ion (salt) bonds. Others form salt bonds with polar lipid regions through interaction with Mg^{+2} or Ca^{+2} ions. However, the interaction of most proteins with lipids in membranes is based on hydrophobic bonds.

Membrane proteins consist of areas rich in polar (charge-bearing) amino acids, for example, lysine (+), arginine (+), glutamic acid (-) and asparagic acid (-), and areas rich in non-polar amino acids (alanine, valine, leucine). Such proteins are located in lipid layers of membranes so that their non-polar areas are embedded in the hydrophobic part of the membrane with hydrophobic lipid areas. The polar (hydrophilic) part of such proteins interacts with lipid heads and is oriented towards the aqueous phase. These are so-called integral proteins and they can only be isolated by destroying the membrane (*fig. 4.5*, 1, 2, 3).

The size of integral membrane proteins is on average 8 nm, but some large proteins – up to 35 nm in size (tilacoid protein in chloroplasts) are also encountered. Typically, these proteins are very asymmetric and, accordingly, are asymmetrically localized in the membrane: their different functional parts are localized on both sides of the membrane, and all proteins of this type are located uniformly. The second group includes semi-integral proteins, which extend only halfway through the membranes on either side (*fig. 4.5*, 4). On the cytoplasmic side of the membrane, integral proteins are connected with another group of proteins, referred to as peripheral proteins (*fig. 4.5*, 5–8). These proteins provide interaction of membranes with supramembrane or submembrane structures. According to their biological role, membrane proteins can be divided into three groups: enzymes, receptor proteins, and structural proteins.

The carbohydrate components in the surface apparatus of cells are present in varying degrees. In animal cells, these are carbohydrates making part of membranes in the form of glycolipids and glycoproteins that are located on the outer surface of the membrane and form the glycocalyx, which is necessary for the cell to communicate with the environment (*fig. 4.6*). Glycocalyx is a layer external to the membrane, 3–4 nm thick, containing oligosaccharide branched chains, which include mannose, glucose, N-acetylglucosamine, sialic acid, etc.

The glycocalyx layer is highly hydrated and has a jelly-like consistency, which significantly reduces the diffusion rate of various substances in this zone. Here, one can also find some hydrolytic enzymes released by the cell that are involved in the extracellular breakdown (extracellular digestion) of polymers to monomer molecules, which are then transported to the cytoplasm through the plasma membrane. In plant cells, the supra-membrane carbohydrate component is more distinct and has the form of the cellulose cell wall.

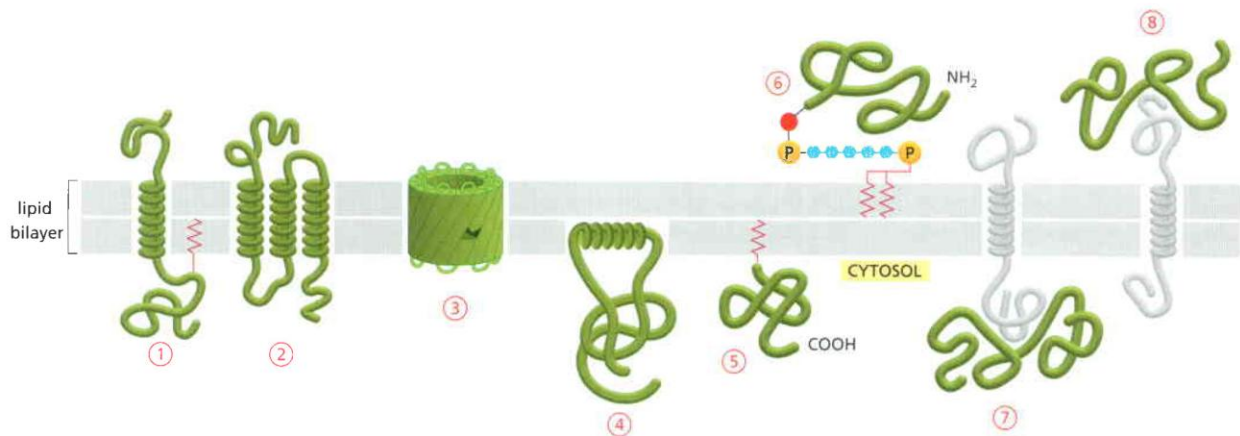


Figure 4.5. Various ways in which membrane proteins associate with the lipid bilayer (Alberts et al., 2008, p. 630)

Most transmembrane proteins are thought to extend across the bilayer as (1) a single α helix, (2) as multiple α helices, or (3) as a rolled-up β sheet (a β barrel). Some of these «single-pass» and «multipass» proteins have a covalently attached fatty acid chain inserted in the cytosolic lipid monolayer (1). Other membrane proteins are exposed at only one side of the membrane. (4) Some of these are anchored to the cytosolic surface by an amphiphilic α helix that partitions into the cytosolic monolayer of the lipid bilayer through the hydrophobic face of the helix. (5) Others are attached to the bilayer solely by a covalently attached lipid chain, either a fatty acid chain or a prenyl group – in the cytosolic monolayer or, (6) via an oligosaccharide linker, to phosphatidylinositol in the noncytosolic monolayer, called a GPI anchor. (7, 8) Finally, many proteins are attached to the membrane only by noncovalent interactions with other membrane proteins

Lipid bilayers are highly impermeable to most polar molecules. To transport small water-soluble molecules into or out of cells or intracellular membrane-enclosed compartments, cell membranes contain various membrane transport proteins, each of which is responsible for transferring a particular solute or class of solutes across the membrane. There are two classes of membrane transport proteins—transporters and channels. Both form continuous protein pathways across the lipid bilayer. Whereas transmembrane movement mediated by transporters can be either active or passive, solute flow through channel proteins is always passive (**fig. 4.7**).

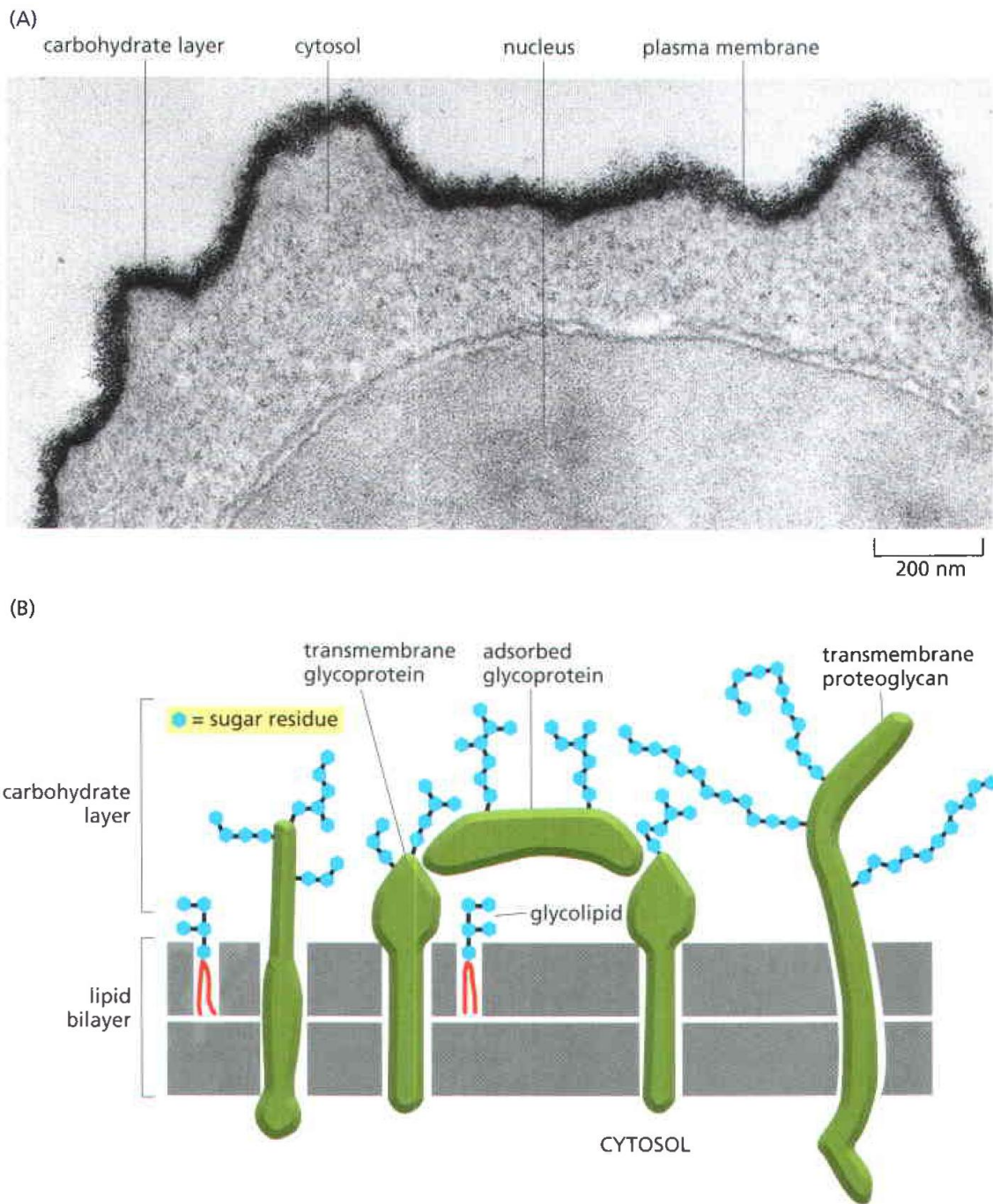


Figure 4.6. The carbohydrate layer on the cell surface (Alberts et al., 2008, p. 637)

A. This electron micrograph of the surface of a lymphocyte stained with ruthenium red emphasizes the thick carbohydrate-rich layer surrounding the cell. (B) The carbohydrate layer is made up of the oligosaccharide side chains of glycolipids and integral membrane glycoproteins and the polysaccharide chains on integral membrane proteoglycans. In addition, adsorbed glycoproteins, and adsorbed proteoglycans (not shown) contribute to the carbohydrate layer in many cells. Note that all of the carbohydrate is on the noncytosolic surface of the membrane

Chapter 4. Cell membranes

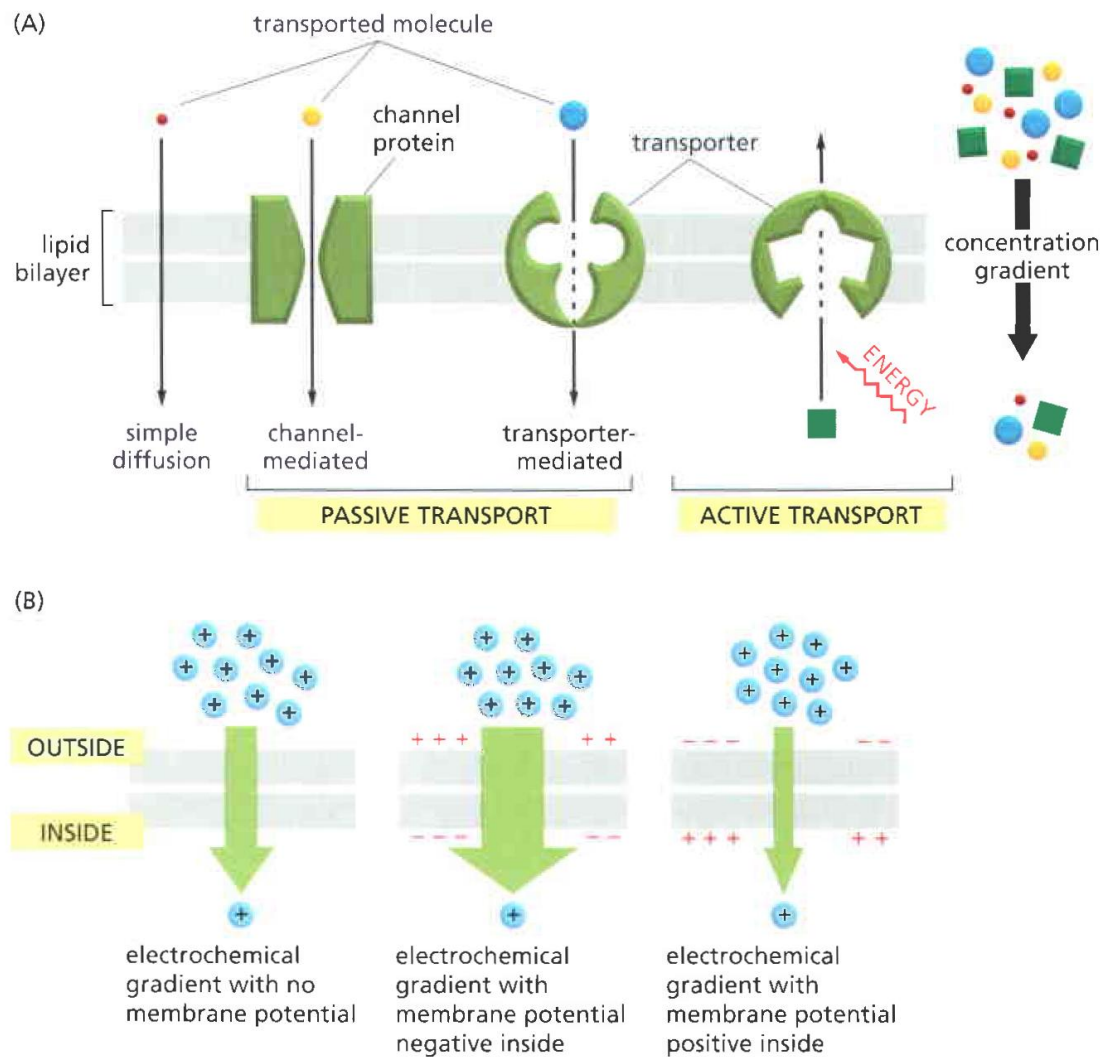


Figure 4.7. Passive and active transport compared (Alberts et al., 2008, p. 654)

(A) Passive transport down an electrochemical gradient occurs spontaneously, either by simple diffusion through the lipid bilayer or by facilitated diffusion through channels and passive transporters. By contrast, active transport requires an input of metabolic energy and is always mediated by transporters that harvest metabolic energy to pump the solute against its electrochemical gradient.

(B) An electrochemical gradient combines the membrane potential and the concentration gradient; they can work additively to increase the driving force on an ion across the membrane (*middle*) or can work against each other (*right*).

Cells carry out such active transport in three main ways (**fig. 4.8**):

1. Coupled transporters couple the uphill transport of one solute across the membrane to the downhill transport of another.
2. ATP-driven pumps couple uphill transport to the hydrolysis of ATP.
3. Light-driven pumps, which are found mainly in bacteria and archaea, couple uphill transport to an input of energy from light.

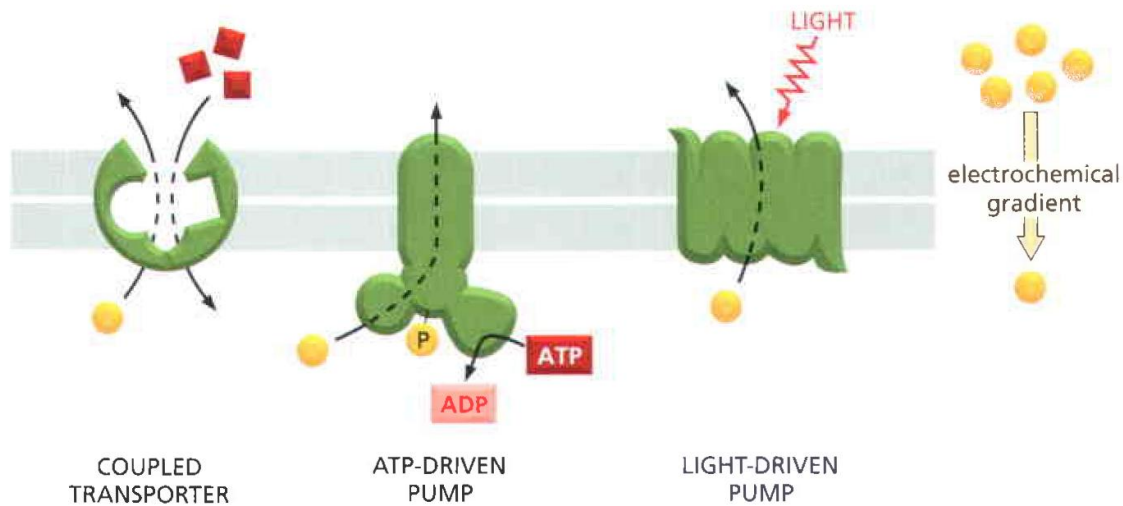


Figure 4.8. Three ways of driving active transport (Alberts et al., 2008, p. 656)
The actively transported molecule is shown in yellow, and the energy source is shown in red.

Some transporters simply mediate the movement of a single solute from one side of the membrane to the other at a rate determined by their V_{max} and K_m ; they are called uniporters. Others function as coupled transporters, in which the transfer of one solute strictly depends on the transport of a second. Coupled transport involves either the simultaneous transfer of a second solute in the same direction, performed by symporters (also called co-transporters), or the transfer of a second solute in the opposite direction, performed by antiporters (also called exchangers) (*fig. 4.9*).

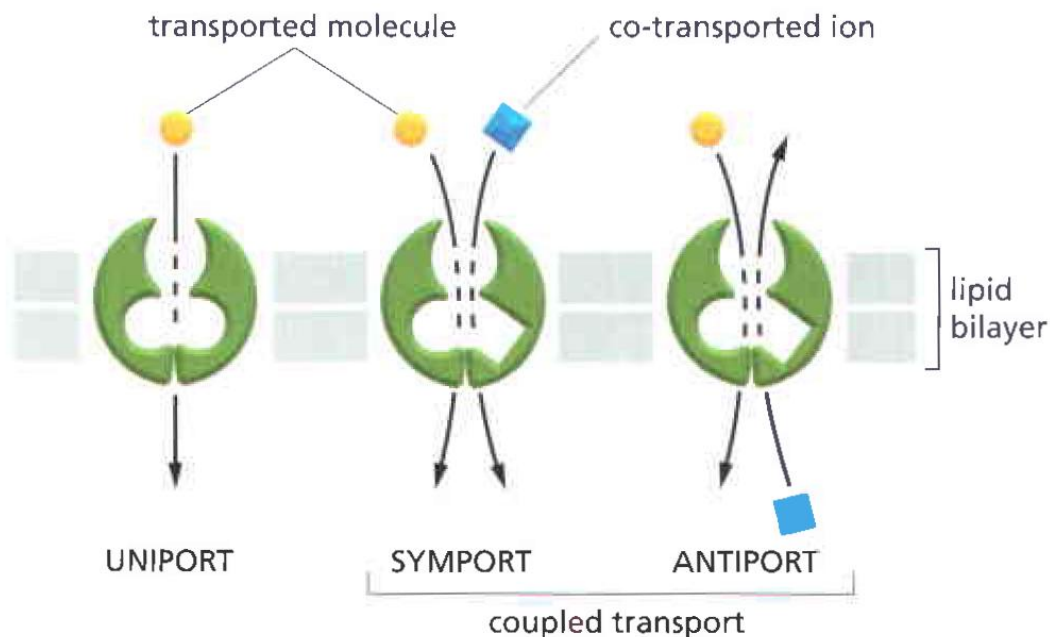


Figure 4.9. Three types of transporter-mediated movement (Alberts et al., 2008, p. 656)
This schematic diagram shows transporters functioning as uniporters, symporters, and antiporters

Transporters bind specific solutes and transfer them across the lipid bilayer by undergoing conformational changes that expose the solute-binding site sequentially on one side of the membrane and then on the other (**fig 4.10**).

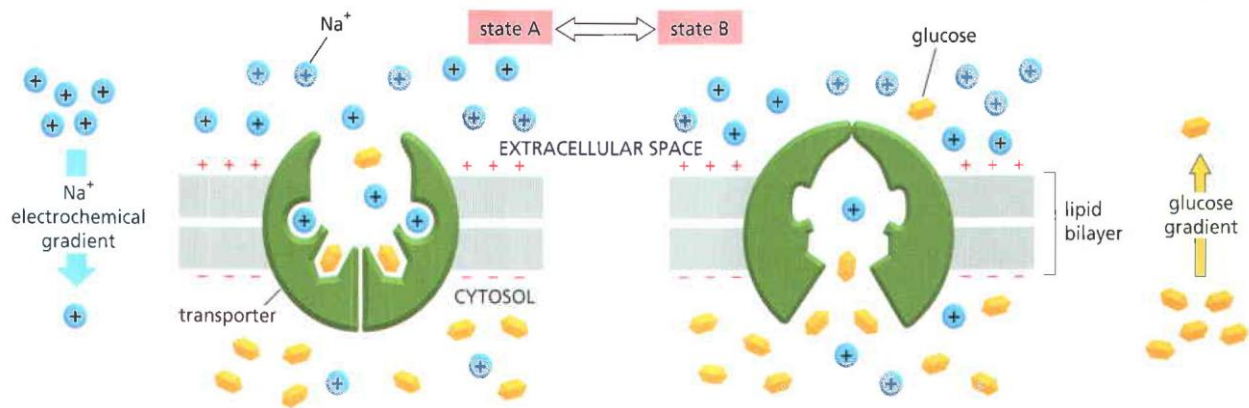


Figure 4.10. One way in which a glucose transporter can be driven by a Na⁺ gradient (Alberts et al., 2008, p. 657)

In the A state, the protein is open to the extracellular space; in the B state, it is open to the cytosol. Binding of Na⁺ and glucose is cooperative, that is, the binding of either ligand induces a conformational change that increases the protein's affinity for the other ligand. Since the Na⁺ concentration is much higher in the extracellular space than in the cytosol, glucose is more likely to bind to the transporter in the A state. Therefore, both Na⁺ and glucose enter the cell (via an A → B transition) much more often than they leave it (via a B → A transition). The overall result is the net transport of both Na⁺ and glucose into the cell. Note that, because the binding is cooperative, if one of the two solutes is missing, the other fails to bind to the transporter. Thus, the transporter undergoes a conformational switch between the two states only if both solutes or neither are bound.

Transporters belong to a small number of protein families. Each family contains proteins of similar amino acid sequences that are thought to have evolved from a common ancestral protein and to operate by a similar mechanism. The family of P-type transport ATPases, which includes Ca²⁺ and Na⁺-K⁺ pumps, is an important example; each of these ATPases sequentially phosphorylates and dephosphorylates itself during the pumping cycle. The superfamily of ABC transporters is the largest family of membrane transport proteins and is especially important clinically (**fig 4.11**).

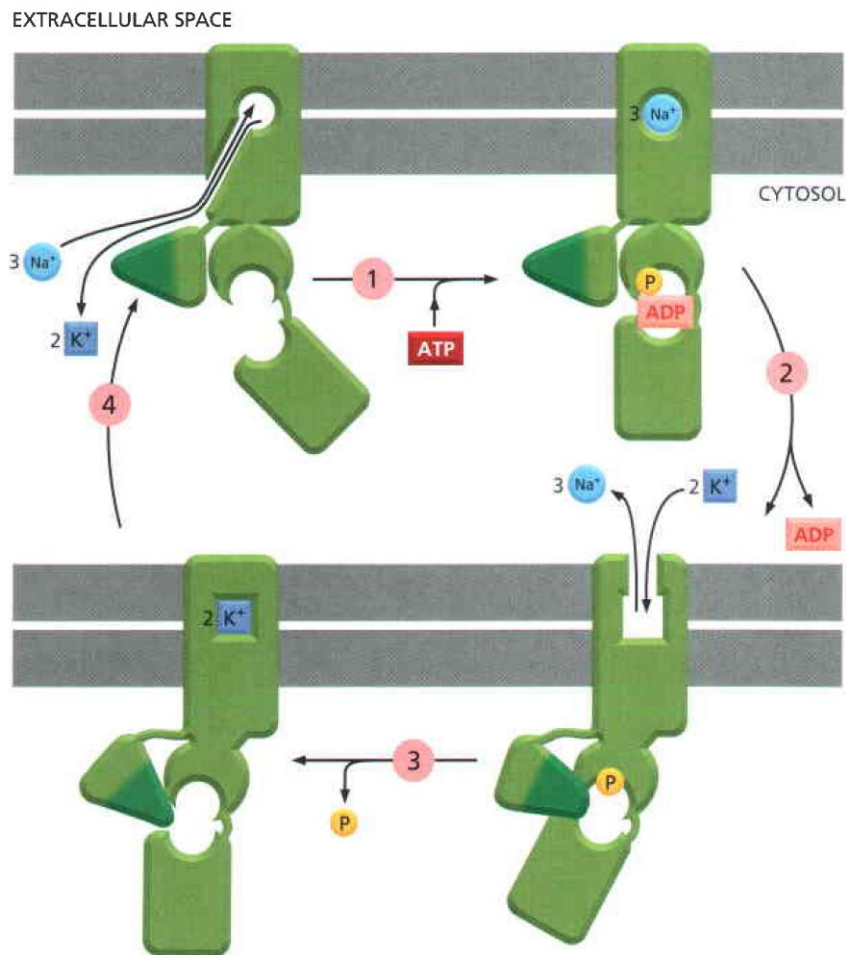


Figure 4.11. A model of the pumping cycle of the Na⁺-K⁺ pump (Alberts et al., 2008, p. 662)

(1) The binding of intracellular Na⁺ and the subsequent phosphorylation by ATP of the cytoplasmic face of the pump induce a conformational change in the protein that (2) transfers the Na⁺ across the membrane and releases it on the outside of the cell. (3) Then, the binding of K⁺ on the extracellular surface and the subsequent dephosphorylation of the pump return the protein to its original conformation, which (4) transfers the K⁺ across the membrane and releases it into the cytosol. The Na⁺- dependent phosphorylation and the Independent dephosphorylation of the protein cause the conformational transitions to occur in an orderly manner, enabling the protein to do useful work. Although for simplicity the diagram shows only one Na⁺- and one K⁺-binding site, in the real pump there are three Na⁺- and two K⁺-binding sites

CONTROL TESTS

Read attentively each assignment (question) and choose one correct answer from the choices offered below.

1. Name the chemical compounds whose molecules mainly provide membrane fluidity

- a) phospholipids;
- b) oligosaccharides;
- c) ATP.

2. Lipids in membranes are mainly represented by:

- a) phospholipids, sphingomyelins and steroids;
- b) phospholipids and steroid lipids;
- c) phospholipids, glycerophosphatides and glycerolipids.

3. Phospholipids are:

- a) complex esters of triatomic alcohol glycerin with two fatty acids and phosphoric acid;
- b) simple esters of glycerin with two fatty acids and phosphoric acid;
- c) lipid compounds with choline.

4. Polar lipid regions carry:

- a) a negative charge;
- b) a positive charge;
- c) no charges.

TOPICS FOR INDEPENDENT WORK

Independent work includes the study of the following topics:

Topic 1: Composition, structure and properties of the lipid bilayer, the structural base of cell membranes.

Topic 2: Barrier and transport functions of plasma membranes. Passive and active transport mechanisms. Gating.

Topic 3: Receptor function of membranes. Adenylate cyclase system.

Topic 4: Vesicular transport: endocytosis and exocytosis.

CYTOSKELETON AND MEMBRANE CONTACT FUNCTION

The concept of the cytoskeleton was first formulated by Nikolai Koltsov, an outstanding Russian biologist, the founder of the Russian school of experimental biology in the early twentieth century. Some elements of cytoskeleton are present in all eukaryotic cells without exception; analogues of these fibrillar structures are also found in prokaryotes. The degree of their expression in different cells may differ. Cytoskeleton cells share some common properties: they are non-branching fibrillar protein polymers, they are unstable, capable of polymerization and depolymerization. The cytoskeleton, or the cell's system of fibers that function in cell support and movement, combines three subsystems that differ in their chemical composition, ultrastructure, and functional properties: the actin filament system; the microtubule system; and the intermediate filament system.

5.1. Actin filament system

Actin is a heterogeneous protein, there may be different variants or isoforms of actin in different cells, and each of them is encoded by its own gene. Actin has a molecular weight of about 42 000 Da and contains an ATP molecule. In its monomeric form, it has the shape of a globule, therefore, it is called G-actin. Actin polymerizes to form a thin fibril termed F-actin, which is a helical ribbon 8 nm thick. Actin microfilaments are polar in their properties. They may be assembled and disassembled depending on the presence of globular actin in the cell (*fig. 5.1*).

A special role in the interaction with actin is performed by myosin-type proteins, which form an actin-myosin complex capable of contracting during ATP breakdown. Myosins are a family of structurally similar proteins. All of them have a head (motor) part responsible for the ATPase-associated activity of the complex, a neck, which is connected with several regulatory protein subunits, and a tail, which is peculiar for each type of myosin that determines its specific function in the cell. There are three main myosin types (*fig. 5.2*).

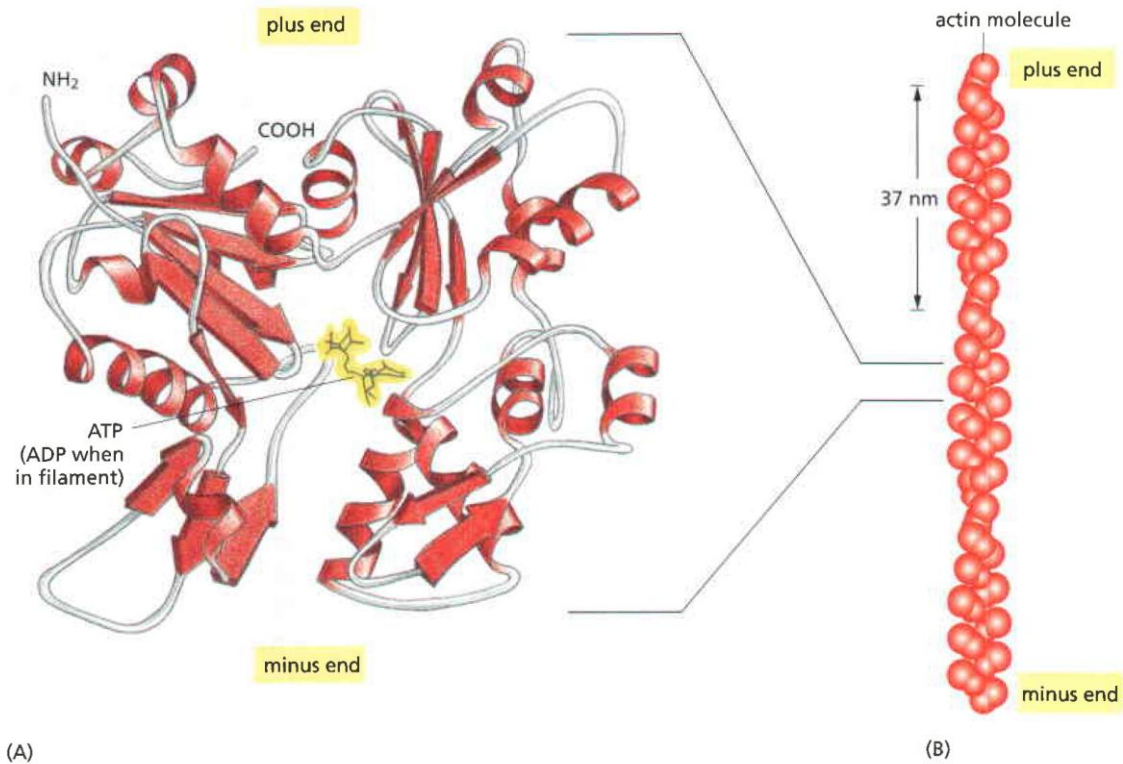


Figure 5.1. The structures of an actin monomer and actin filament (Alberts et al., 2008, p. 975)

(A) The actin monomer has a nucleotide (either ATP or ADP) bound in a deep cleft in the center of the molecule. (B) Arrangement of monomers in a filament. Although the filament is often described as a single helix of monomers, it can also be thought of as consisting of two protofilaments, held together by lateral contacts, which wind around each other as two parallel strands of a helix, with a twist repeating every 37 nm. All the subunits within the filament have the same orientation

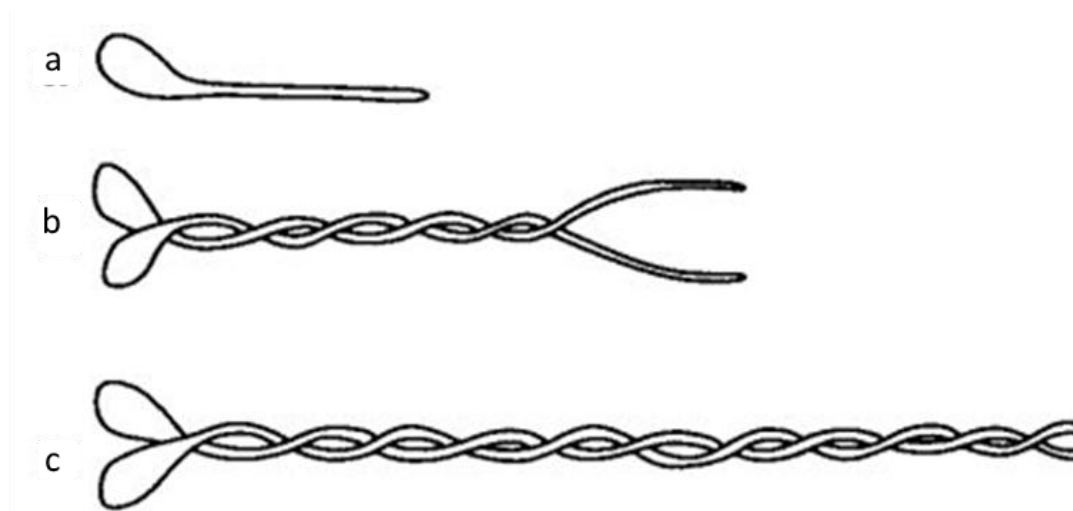


Figure 5.2. Myosin types (Chentsov, 2015, p. 384)

a – myosin I; b – myosin V; c – myosin II

Myosin I is a monomer molecule. Myosin II and myosin V are dimers in which the α -helix tail forms a coiled-coil rod portion. Two molecules of myosin II can associate with each other, forming a bipolar thick fibril, which participates in muscle contraction, in the contraction of intracellular microfilament bundles, and in cell division.

Type I and type V myosins participate in interactions between cytoskeleton and membrane elements, in the transport of secretory vesicles. Myosin, which is a component of thick threads, is a large protein with a molecular weight of 500 thousand. It consists of six chains: two long chains spirally wrapped around one another (heavy chains), and four short chains (light chains), which bind to the globular heads of long chains. The association of myosin heads with actin produces actin-myosin complexes whose activity is hundreds of times higher than the ATP activity of myosins alone.

The thickness of myosin fibrils is due to the fact that long (150 nm) myosin molecules aggregate so that they form bundles that include about 300 such molecules. In thick myosin protofibrils, long molecules form a «tail-to-tail» arrangement so that myosin heads are located at the ends of such threads, and there are no heads in their middle part (*fig. 5.3*). The heads form cross-bridges linking actin and myosin filaments.

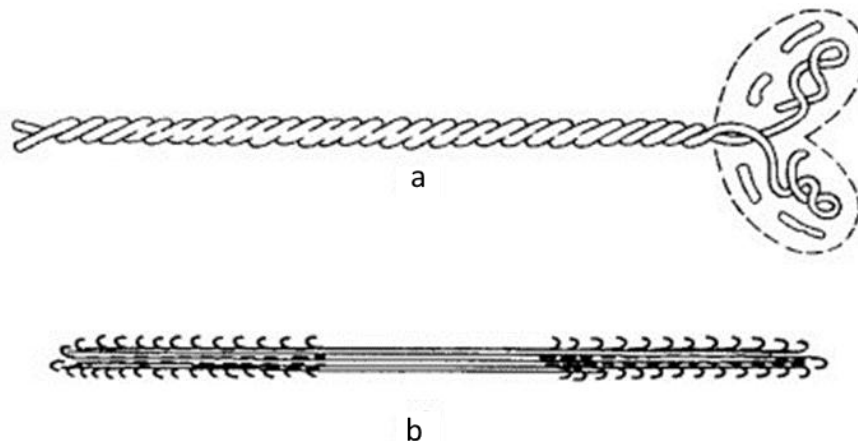


Figure 5.3. Myosin molecule (a) and myosin protofilament (b) (Chentsov, 2015, p. 390)

Regardless of myosin type, the mechanism of action of actin-myosin complexes is very similar: it begins when the myosin head attaches to the actin filament, then bends and subsequently detaches from the actin filament (*fig. 5.4*).

In each cycle, the myosin head moves 5–25 nm in the direction of the plus-end of the actin filament during hydrolysis of one ATP molecule. Thus, the microfilaments move or slide unidirectionally relative to myosin molecules.

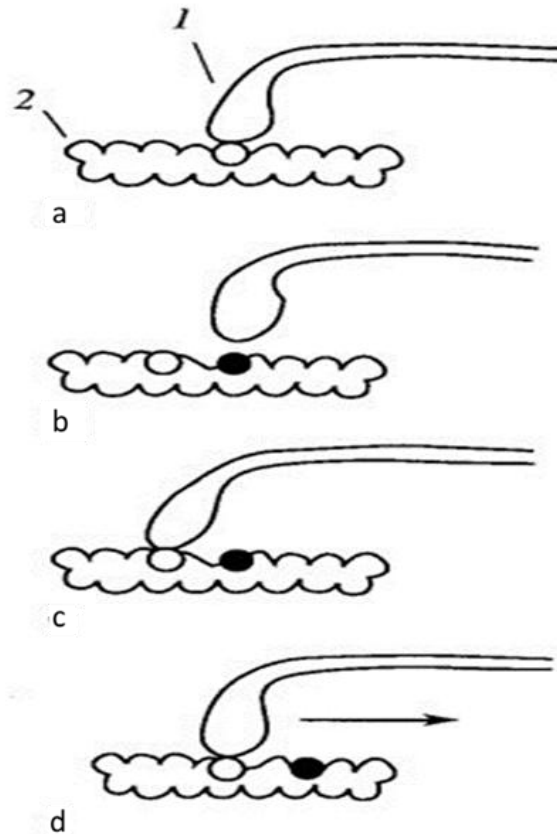


Figure 5.4. The actin-myosin movement sequence (Chentsov, 2015, p. 384)

a – starting position: myosin head (1) is attached to actin filament (2); b – myosin head moves away from actin filament; c – myosin head is attached to another actin subunit; d – myosin head moves to the starting position: actin filament translocation

Let us consider the work of contracting cell structures using the sliding filament model proposed by British scientists Hanson and Huxley (1954). The basic subunit within myofibrils is a sarcomere, an area between two Z-disks. The size of sarcomeres in a relaxed state is always the same (1.8–2.8 μm depending on the animal species). There are three areas of protofibrils along the sarcomere: light, connected to the Z-disk, then dark and again light, connected to the next Z-disk.

Contraction occurs due to a reduction in the distance between the Z-discs, i.e. a shortening of the sarcomeres by about 20%. The mechanism of muscle contraction is a cooperative shortening of all sarcomeres along the entire length of myofibrils. The contraction has been found to be based on the movement of light and dark filaments relative to each other. In this case, thick myosin filaments get into the spaces between the actin filaments, bringing Z-drives closer to each other (**fig. 5.5**).

Smooth muscle cells also have actin and myosin filaments, but they are not positioned as regularly as in striated muscle tissue. There are no sarcomeres, and myosin molecules are located without any special order among the actin protofibril bundles. They form complexes of 15–20 myosin molecules rather than thick aggregates (**fig. 5.6**).

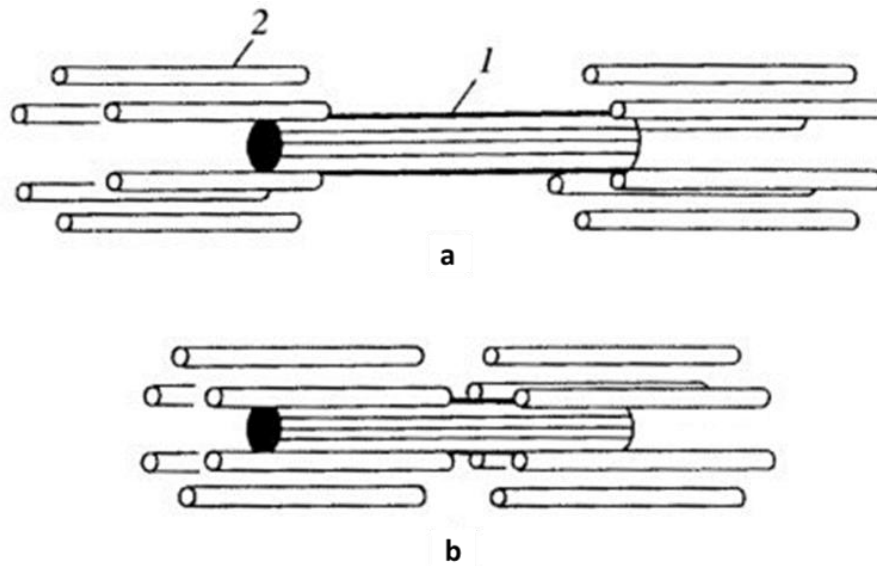


Figure 5.5. Schematic of muscle contraction due to mutual sliding of filaments (Chentsov, 2015, p. 391)

a – relaxation; b – contraction; 1 – thick filaments (myosin); 2 – thin filaments (actin)

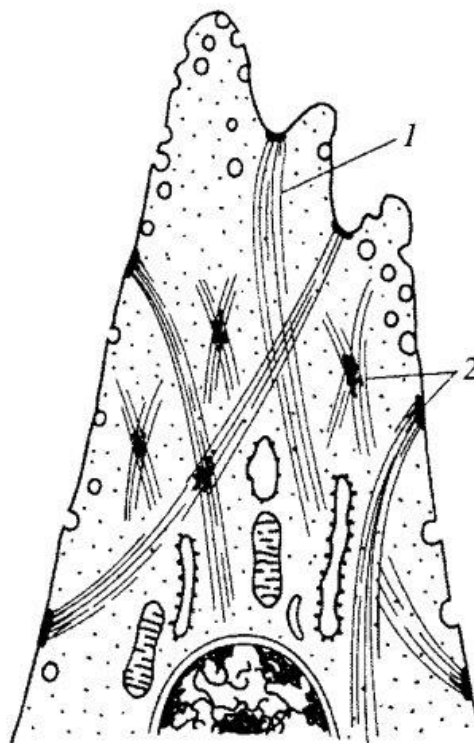


Figure 5.6. Schematic of smooth muscle cell structure (Chentsov, 2015, p. 392)

1 – actin-myosin bundles; 2 – dense membrane and cytoplasmic bodies

5.2. Tubulin-microtubule system

The tubulin system has much in common with the microfilament system: first, the ability to polymerize and depolymerize; second, the presence of polarity in the protein filaments, and third, a large number of auxiliary proteins. The main protein of microtubules is tubulin. The tubulin molecule is a heterodimer, consisting of two different subunits, α -tubulin and β -tubulin, which associate to form the tubulin protein. During polymerization, tubulin molecules are combined in such a way that the β -subunit of one protein is associated with the α -subunit of the next protein, etc., thus forming protofilaments. During polymerization, tubulin molecules form 13 longitudinal protofilaments, which are twisted into a hollow tube, so one can see 13 globular molecules in the cross section of the microtubule. Tubulin monomer size is about 5 nm, so the microtubule wall thickness is also 5 nm. Morphologically, microtubules are long hollow cylinders with an outer diameter of 25 nm (**fig. 5.7**).

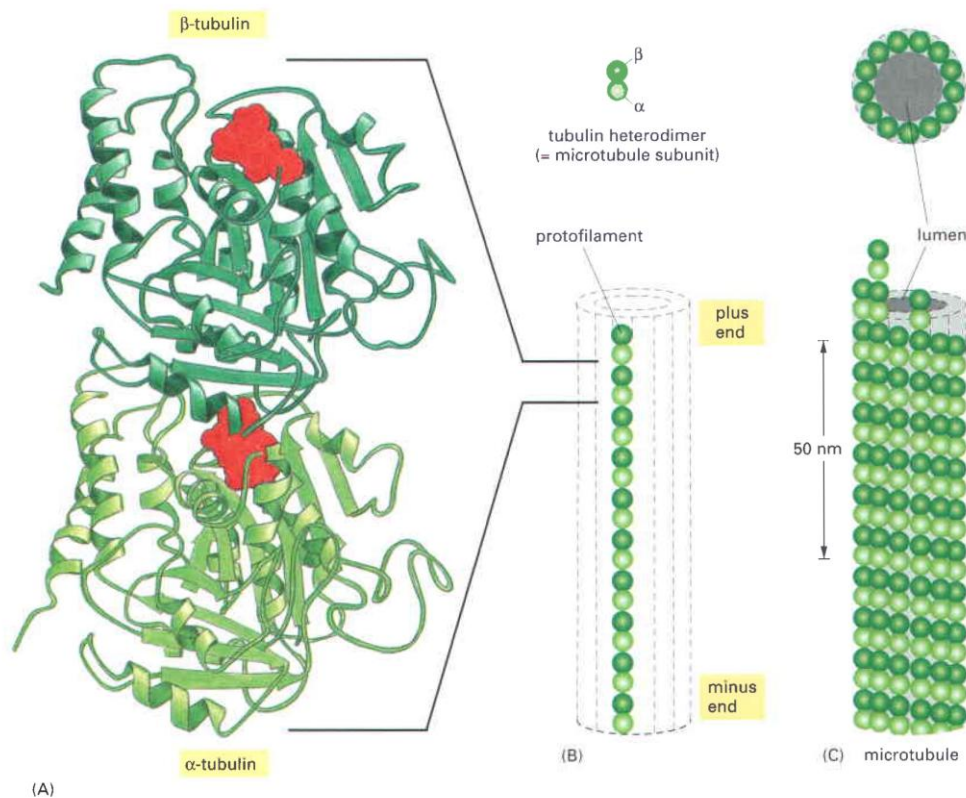


Figure 5.7. The structure of a microtubule and its subunit (Alberts et al., 2008, p. 974)

(A) The subunit of each protofilament is a tubulin heterodimer, formed from a very tightly linked pair of α - and β -tubulin monomers. The GTP molecule in the α -tubulin monomer is so tightly bound that it can be considered an integral part of the protein. The GTP molecule in the β -tubulin monomer, however, is less tightly bound and has an important role in filament dynamics. Both nucleotides are shown in red. (B) One tubulin subunit (α - β heterodimer) and one protofilament are shown schematically. Each protofilament consists of many adjacent subunits with the same orientation. (C) The microtubule is a stiff hollow tube formed from 13 protofilaments aligned in parallel

Microtubules are very dynamic structures that can assemble and disassemble quite quickly. With the sufficient tubulin concentration, polymerization occurs spontaneously. During the extension of the length of the microtubule, the binding of the tubules occurs at a higher rate at the growing (+) end. However, if the concentration of tubulin is insufficient, the microtubules can disassemble at both ends. Disassembly of microtubules is facilitated by a decrease in temperature and the presence of Ca^{+2} ions.

The average life duration of cytoplasmic microtubules is only 5 minutes. In about 15 minutes, approximately 80% of the entire population of microtubules is renewed. In this process, individual microtubules can slowly (4–7 $\mu\text{m}/\text{min}$) lengthen at their growing end and then can shorten rather quickly (14–17 $\mu\text{m}/\text{min}$). The assembly and disassembly of microtubules is continuous and this process reflects the main characteristic of microtubules, their dynamic instability.

It is interesting to note that microtubules in the division spindle have a lifetime of about 15–20 seconds. It is believed that dynamic instability of cytoplasmic microtubules is related to the delay of guanosine triphosphate (GTP) hydrolysis. Stabilization of microtubules is due to modification of tubulins, for example, acetylation of lysine in tubulins considerably increases microtubules stability. Another example of tubulin modification is the removal of terminal tyrosine, which is also typical for microtubule stabilization. These modifications are reversible.

Microtubules themselves are not capable of contraction, but they are obligatory components of many moving cell structures, such as cilia and flagella, cell spindles during mitosis and microtubules of cytoplasm, which are obligatory for a number of intracellular transport types, such as exocytosis, movement of intracellular structures (mitochondria, secretory vesicles, etc.).

Microtubules begin their growth from special sites – microtubule organizing centers (MTOCs). The growth of cytoplasmic microtubules is polar: the plus end of microtubules extends. In animal cells, the cell center acting as a MTOC is the centrosome represented by two perpendicularly arranged centrioles, which in turn consist of a system of organized microtubules, and a pericentriolar matrix. In higher plant cells that have no cell centers, microtubule polymerization occurs at the periphery of the cell nucleus, from which microtubules extend radially.

Microtubules' minus ends are turned to the MTOCs, where they are blocked by special proteins that prevent or limit the depolymerization of tubulins. The motor role of microtubules is that they create an ordered, vectored motion system. Cytoplasmic microtubules together with specific associated motor proteins (kinesins and dyneins) form ATPase complexes capable of driving cellular

components. Cytoplasmic dyneins and kinesins have been found in almost all types of animal and plant cells.

Kinesin is a protein that has a molecular weight of about 300 thousand. It consists of two similar heavy polypeptide chains and several light ones. Each heavy chain forms a globular head which, when associated with a microtubule, displays ATPase activity, while the light chains bind to the vesicle membrane. During ATP hydrolysis, the conformation of the kinesin molecule changes and the movement of the particle towards the plus end of the microtubule is generated.

There is a whole family of kinesins with similar motor heads but different tail domains. For example, cytosolic kinesins are involved in the transport of secretory vesicles, lysosomes, mitochondria, synaptic vesicles via microtubules. Kinesins bind to the membranes of their cargo structures through membrane protein complexes – kinectins. Mitotic spindle kinesins participate both in the formation of the structure itself, and in the movement of chromosomes to the poles of the cell. Thus, the pattern of movement in cytoplasm is similar to the sliding filaments principle, but instead of filaments, short molecules (motor proteins associated with moving cellular components) travel along microtubules.

The action of microtubules is similar to that of the actin-myosin complex. In this case, a double complex (microtubule + proteins) with a high ATPase activity is formed.

5.3. Intermediate filament system

Intermediate filaments are the third important component of cytoskeleton. These are the most stable and long-lasting cytoskeleton elements. They serve as a support system in cells exposed to significant physical load. In skin epidermis cells, intermediate filaments form bundles (tonofilaments) connected to mechanical contacts (desmosomes) and create a rigid intracellular network (**fig. 5.8**). In terms of cell topography, the location of intermediate filaments follows that of microtubules, they are juxtaposed.

In terms of their properties and functions, cytoskeleton elements can be divided into two groups: fibrils that perform only the role of the scaffold (intermediate filaments), motor fibrils (actin microfilaments that interact with motor proteins, myosins), and tubulin microtubules that interact with motor proteins, dyneins, and kinesins.

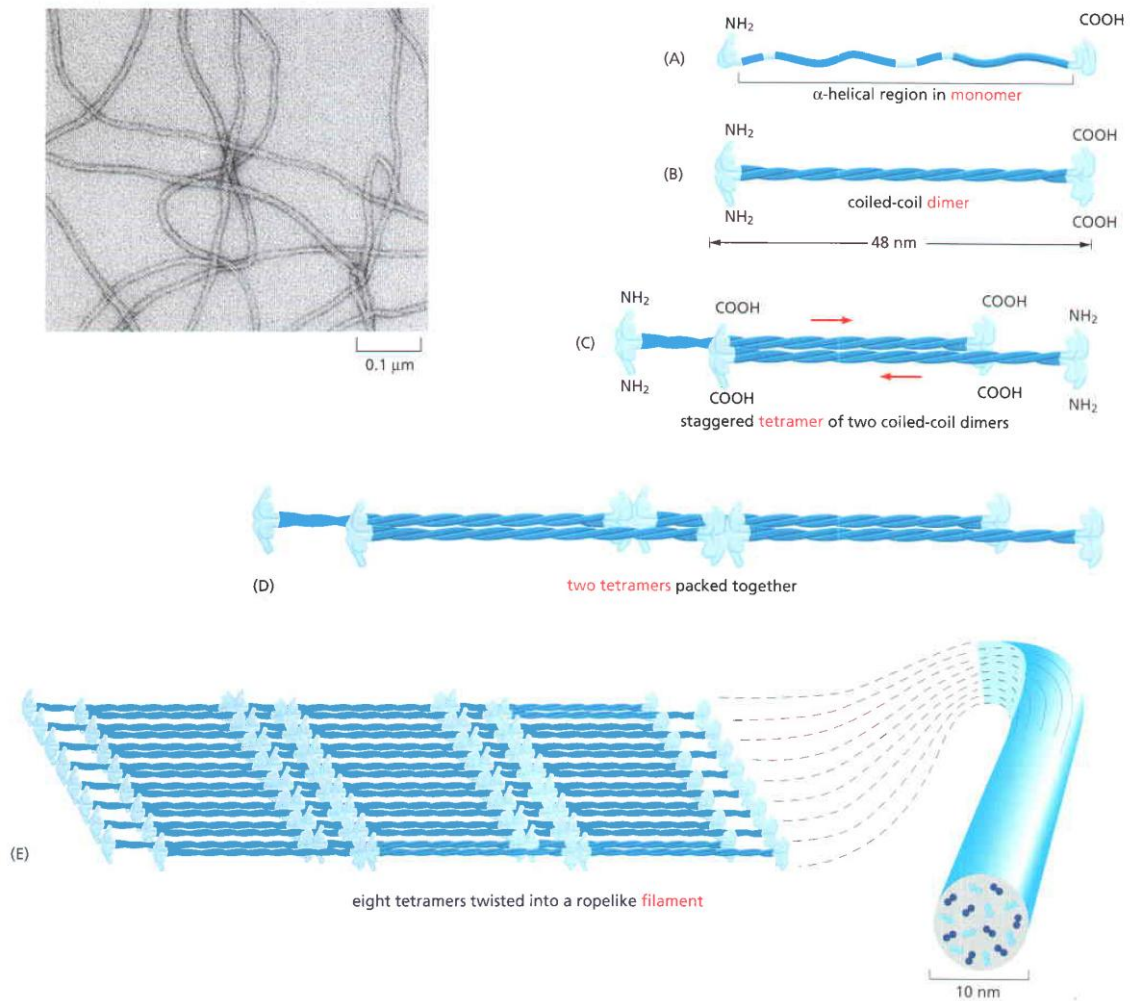


Figure 5.8. A model of intermediate filament construction (Alberts et al., 2008, p. 984)

The monomer shown in (A) pairs with an identical monomer to form a dimer (B), in which the conserved central rod domains are aligned in parallel and wound together into a coiled coil. (C) Two dimers then line up side by side to form an antiparallel tetramer of four polypeptide chains. The tetramer is the soluble subunit of intermediate filaments. (D) Within each tetramer, the two dimers are offset with respect to one another, thereby allowing it to associate with another tetramer. (E) In the final 10-nm rope-like filament, tetramers are packed together in a helical array, which has 16 dimers (32 coiled coils) in cross-section. Half of these dimers are pointing in each direction. An electron micrograph of intermediate filaments is shown on the upper left (Electron micrograph courtesy of Roy Quinlan)

CONTROL TESTS

Read attentively each assignment (question) and choose one correct answer from the choices offered below.

1. The main protein in the eukaryotic cell cytoskeleton microfilament system is:

- a) actin;
- b) tubulin;
- c) filamin.

2. In cells, actin protein molecules may be in two states:

- a) in a monomeric and a polymeric form;
- b) in spiralized and diffused state;
- c) in the form of primary and secondary structures.

3. Proteins that together with actin form a complex capable of contraction when ATP breaks down are called:

- a) myosins;
- b) filamins;
- c) fimbrins.

4. In each cycle, the myosin head moves in the direction of the plus-end of the actin filament during hydrolysis of one ATP molecule to a distance of

- a) 5–25 nm;
- b) 1–4 nm;
- c) 30–45 nm.

TOPICS FOR INDEPENDENT WORK

Independent work includes the study of the following topics:

Topic 1: Submembrane system of the hyaloplasm. Peripheral hyaloplasm and the structured support and contraction system (cytoskeleton).

Topic 2: The system of intermediate and thin filaments. Their function and structure.

Topic 3: Contact function of the plasma membrane. Intercellular junctions.

Topic 4: Adherent (mechanical) junctions: belt desmosomes, point desmosomes and hemidesmosomes.

Topic 5: Occluding junctions: tight, intermediate (fusion zone).

Topic 6: Communicating junctions: gap junction, chemical synapses and plasmodesmata.

VACUOLAR SYSTEM

The main inner cell medium (hyaloplasm) is filled with a complex three-dimensional network called the vacuolar system. It consists of single-membrane organoids that have different structure and functions (endoplasmic reticulum, Golgi complex, lysosomes, endosomes, peroxisomes, secretory vacuoles). The vacuolar system is characterized by cooperative functioning and a particular sequence of the stages of formation, reorganization, transport and export of synthesized products.

The vacuolar system performs:

1. Mechanical functions by dividing the cell hyaloplasm into compartments.
2. The function of regulation of metabolism between internal and external components of the cell.
3. The function of synthesis, rearrangement, sorting and export of biopolymers, mainly glycoproteins, from the cell.

6.1. Endoplasmic reticulum

All eukaryotic cells have an endoplasmic reticulum (ER). Its membrane typically constitutes more than half of the total membrane of an average animal cell. The ER is organized into a netlike labyrinth of branching tubules and flattened sacs that extends throughout the cytosol (**fig. 6.1**). The tubules and sacs

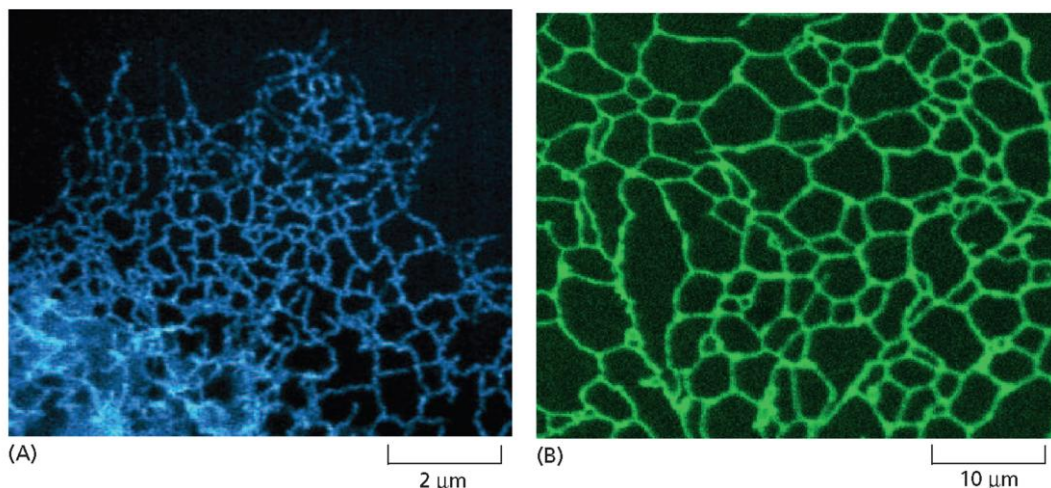


Figure 6.1. Fluorescent micrographs of the endoplasmic reticulum (Alberts et al., 2008, p. 724)

(A) Part of the ER network in a cultured mammalian cell, stained with an antibody that binds to a protein retained in the ER. The ER extends as a network throughout the entire cytosol, so that all regions of the cytosol are close to some portion of the ER membrane. (B) Part of an ER network in a living plant cell that was genetically engineered to express a fluorescent protein in the ER. (A, courtesy of Hugh Pelham; B, courtesy of Petra Boevink and Chris Hawes.)

interconnect, and their membrane is continuous with the outer nuclear membrane. Thus, the ER and nuclear membranes form a continuous sheet enclosing a single internal space, called the ER lumen or the ER cisternal space, which often occupies more than 10% of the total cell volume.

The ER has a central role in both lipid and protein biosynthesis, and it also serves as an intracellular Ca^{2+} store that is used in many cell signaling responses.

There are two types of ER: rough and smooth (**fig. 6.2**).

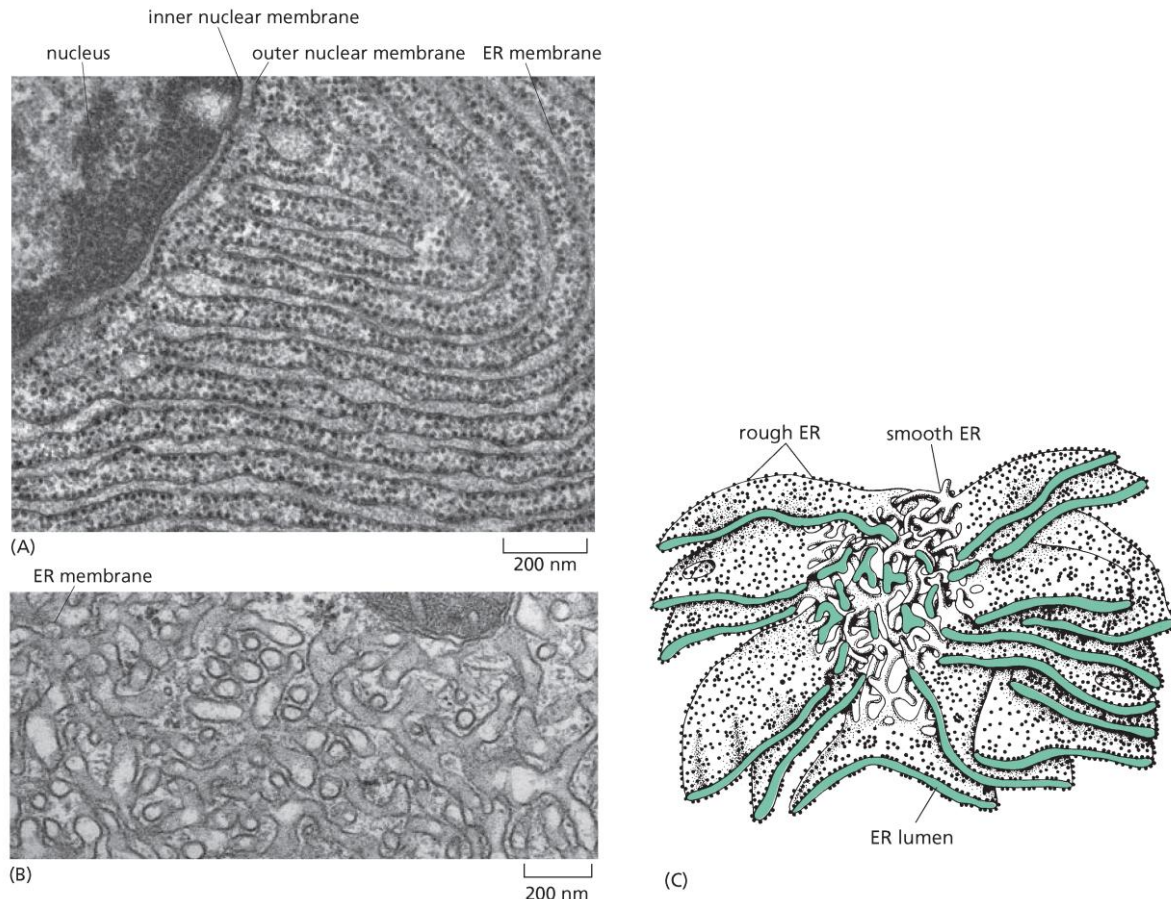


Figure 6.2. The rough and smooth ER (Alberts et al., 2008, p. 725)

(A) An electron micrograph of the rough ER in a pancreatic exocrine cell that makes and secretes large amounts of digestive enzymes every day. The cytosol is filled with closely packed sheets of ER membrane studded with ribosomes. At the top left is a portion of the nucleus and its nuclear envelope; note that the outer nuclear membrane, which is continuous with the ER, is also studded with ribosomes. (B) Abundant smooth ER in a steroid-hormone-secreting cell. This electron micrograph is of a testosterone-secreting Leydig cell in the human testis. (C) A three-dimensional reconstruction of a region of smooth ER and rough ER in a liver cell. The rough ER forms oriented stacks of flattened cisternae, each having a luminal space 20–30 nm wide. The smooth ER membrane is connected to these cisternae and forms a fine network of tubules 30–60 nm in diameter. (A, courtesy of Lelio Orci; B, courtesy of Daniel S. Friend; C, after R.V. Krstic, *Ultrastructure of the Mammalian Cell*. New York: Springer-Verlag, 1979.)

Granular or rough endoplasmic reticulum (ER) is made up of closed membranes which form elongated sacs (cisternae) or have the shape of narrow channels. The width of cisternal cavities may vary greatly depending on the

functional activity of the cell. A distinctive feature of these membranes is that they are covered with ribosomes on the hyaloplasmic side. These ribosomes' function is to synthesize protein. The number of ribosomes on ER is closely related to its synthetic activity. The ribosomes attached to the rough ER synthesize proteins to be «exported» from a given cell. Let us consider the synthesis of soluble proteins (*fig. 6.3*).

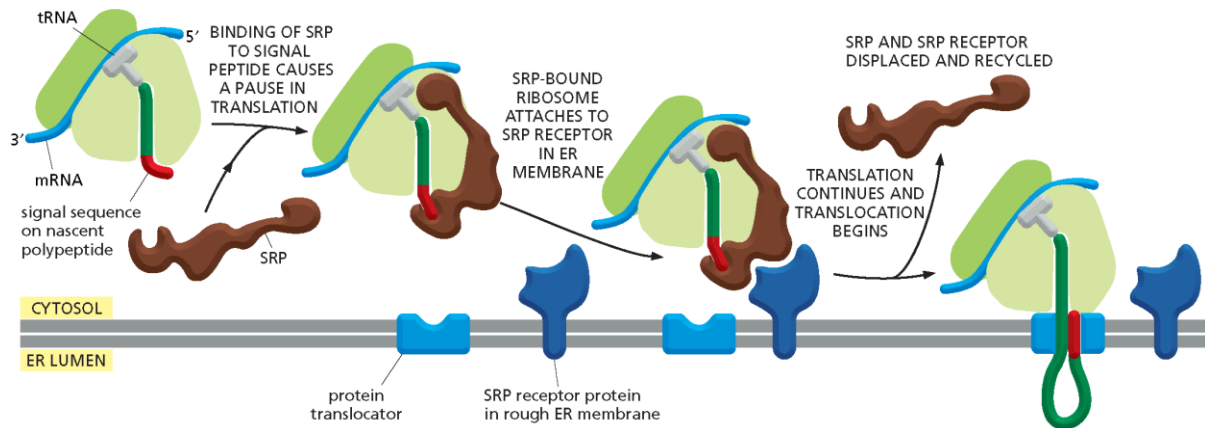


Figure 6.3. How ER signal sequences and SRP direct ribosomes to the ER membrane (Alberts et al., 2008, p. 729)

The SRP and its receptor are thought to act in concert. The SRP binds to both the exposed ER signal sequence and the ribosome, thereby inducing a pause in translation. The SRP receptor in the ER membrane, which is composed of two different polypeptide chains, binds the SRP-ribosome complex and directs it to the translocator. In a poorly understood reaction, the SRP and SRP receptor are then released, leaving the ribosome bound to the translocator in the ER membrane. The translocator then inserts the polypeptide chain into the membrane and transfers it across the lipid bilayer. Because one of the SRP proteins and both chains of the SRP receptor contain GTP-binding domains, it is thought that conformational changes that occur during cycles of GTP binding and hydrolysis ensure that SRP release occurs only after the ribosome has become properly engaged with the translocator in the ER membrane. The translocator is closed until the ribosome has bound, so that the permeability barrier of the ER membrane is maintained at all times

The ER signal sequence is guided to the ER membrane by at least two components: a signal-recognition particle (SRP), which cycles between the ER membrane and the cytosol and binds to the signal sequence, and an SRP receptor in the ER membrane. The SRP is a complex particle, consisting of six different polypeptide chains bound to a single small RNA molecule. SRP and its receptor are found in all cells, indicating that this protein-targeting mechanism arose early in evolution and has been conserved. The SRP is a rodlike structure that wraps around the large ribosomal subunit, with one end binding to the ER signal sequence as it emerges as part of the newly made polypeptide chain from the ribosome; the other end blocks the elongation factor binding site at the interface between the large and small ribosomal subunits. This block halts protein synthesis as soon as the signal peptide has emerged from the ribosome. The transient pause

presumably gives the ribosome enough time to bind to the ER membrane before completion of the polypeptide chain, thereby ensuring that the protein is not released into the cytosol. This safety device may be especially important for secreted and lysosomal hydrolases that could wreak havoc in the cytosol; cells that secrete large amounts of hydrolases, however, take the added precaution of having high concentrations of hydrolase inhibitors in their cytosol.

Once formed, the SRP-ribosome complex binds to the SRP receptor, which is an integral membrane protein complex embedded in the rough ER membrane. This interaction brings the SRP-ribosome complex to a protein translocator. The SRP and SRP receptor are then released, and the translocator transfers the growing polypeptide chain across the membrane.

This co-translational transfer process creates two spatially separate populations of ribosomes in the cytosol. Membrane-bound ribosomes, attached to the cytosolic side of the ER membrane, are engaged in the synthesis of proteins that are being concurrently translocated into the ER. Free ribosomes, unattached to any membrane, synthesize all other proteins encoded by the nuclear genome. Membrane-bound and free ribosomes are structurally and functionally identical. They differ only in the proteins they are making at any given time.

The proteins thus synthesized undergo various modifications. The most characteristic modification is primary glycosylation, i.e. covalent binding of a protein chain to a complex oligosaccharide. It also occurs in a co-translational way. The oligosaccharide complex contains 2 molecules of N-acetyl glycosamine, 9 molecules of mannose and 3 molecules of glucose and is bound to a special lipid, dolichol, on the inner surface of the membrane facing the lumen of the ER cistern. As the protein chain is translocated during its synthesis, each asparagine residue interacts with the oligosaccharide complex using integral proteins of ER membranes. Both soluble and membrane proteins are subjected to primary glycosylation. As a result, the synthesized protein becomes a glycoprotein.

In addition, in the cavity of ER cisternae, proteins undergo a number of additional changes: disulfide bonds are formed. The proper folding of polypeptide chains takes place. This process is ensured by specific proteins called chaperones. The term «chaperones» was suggested by R. Ellis in 1987. Chaperones have an affinity for hydrophobic parts of polypeptide chains and function to protect proteins from denaturation. Binding to chaperones protects the unfolded polypeptide chain from contacts with other proteins and creates conditions for normal folding of the growing peptide.

In smooth ER membranes, cell membrane lipids, including phospholipids and cholesterol, are synthesized and assembled. Enzymes involved in lipid synthesis are embedded in the ER membrane on its cytosolic side. Thus, the bilipid layer of the membrane grows and increases the surface of the ER cistern. This process occurs simultaneously with the synthesis of integral membrane proteins, so that the lipoprotein membrane is formed and grows in the course of two simultaneous processes: the synthesis and integration of lipids, and the synthesis and integration of membrane proteins.

A major portion of the cell's protein synthesis occurs on the cytosolic surface of the ER: all proteins destined for secretion and all proteins destined for the ER itself, the Golgi apparatus, the lysosomes, the endosomes, and the plasma membrane are first imported into the ER from the cytosol. In the ER lumen, the proteins fold and oligomerize, disulfide bonds are formed, and N-linked oligosaccharides are added. The pattern of N-linked glycosylation is used to indicate the extent of protein folding, so that proteins leave the ER only when they are properly folded. Proteins that do not fold or oligomerize correctly are translocated back into the cytosol, where they are deglycosylated, ubiquitinated, and degraded in proteasomes. If misfolded proteins accumulate in excess in the ER, they trigger an unfolded protein response, which activates appropriate genes in the nucleus to help the ER to cope.

Only proteins that carry a special ER signal sequence are imported into the ER. The signal sequence is recognized by a signal recognition particle (SRP), which binds both the growing polypeptide chain and a ribosome and directs them to a receptor protein on the cytosolic surface of the rough ER membrane. This binding to the ER membrane initiates the translocation process by threading a loop of polypeptide chain across the ER membrane through the hydrophilic pore in a transmembrane protein translocator.

Soluble proteins, destined for the ER lumen, for secretion, or for transfer to the lumen of other organelles, pass completely into the ER lumen. Transmembrane proteins destined for the ER or for other cell membranes are translocated partway across the ER membrane and remain anchored there by one or more membrane-spanning α -helical regions in their polypeptide chains. These hydrophobic portions of the protein can act either as start-transfer or stop-transfer signals during the translocation process.

6.2. Golgi complex

The Golgi complex is an organelle found in most eukaryotic cells. Part of the endomembrane system in the cytoplasm, it packages proteins into membrane-bound vesicles inside the cell before the vesicles are sent to their destination. It resides at the intersection of the secretory, lysosomal, and endocytic pathways. It is of particular importance in processing proteins for secretion, containing a set of glycosylation enzymes that attach various sugar monomers to proteins as the proteins move through the apparatus.

It was identified in 1897 by the Italian scientist Camillo Golgi and was named after him in 1898.

In most eukaryotes, the Golgi apparatus is made up of a series of compartments and is a collection of fused, flattened membrane-enclosed disks (cisterna) known as dictyosome, originating from vesicular clusters that bud off the endoplasmic reticulum (**fig. 6.4**). A mammalian cell typically contains 40 to 100 stacks of cisternae. Between four and eight cisternae are usually present in a stack; however,

in some protists as many as sixty cisternae have been observed. This collection of cisternae is broken down into cis, medial, and trans compartments, making up two main networks: the cis Golgi network (CGN) and the trans Golgi network (TGN). The CGN is the first cisternal structure, and the TGN is the final, from which proteins are packaged into vesicles destined to lysosomes, secretory vesicles, or the cell surface.

In all eukaryotes, each cisternal stack has a cis entry face and a trans exit face. These faces are characterized by unique morphology and biochemistry. Within individual stacks are assortments of enzymes responsible for selectively modifying protein cargo. These modifications influence the fate of the protein. The compartmentalization of the Golgi apparatus is advantageous for separating enzymes, thereby maintaining consecutive and selective processing steps: enzymes catalyzing early modifications are gathered in the cis face cisternae, and enzymes catalyzing later modifications are found in trans face cisternae of the Golgi stacks.

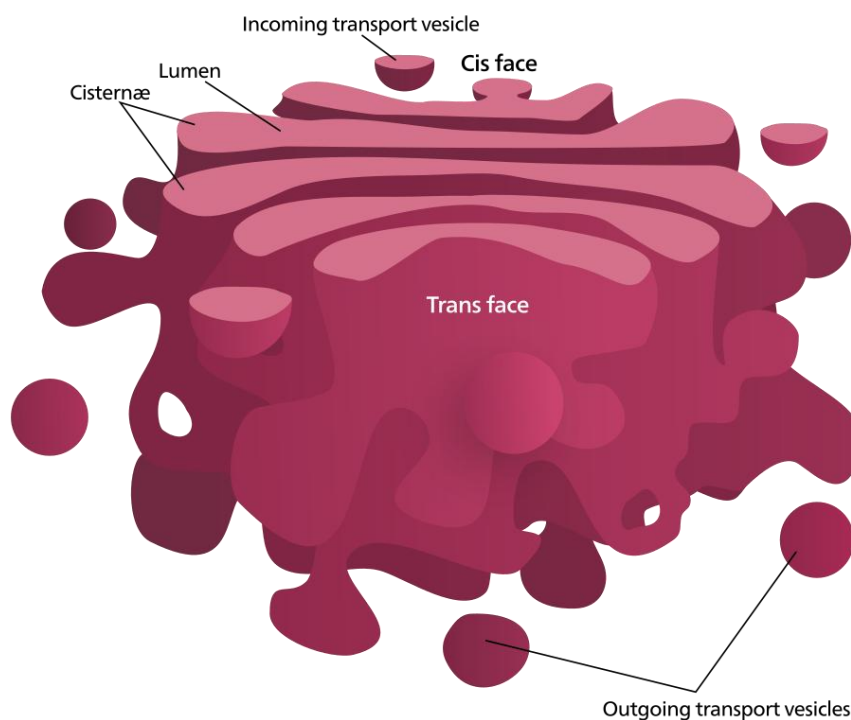


Figure 6.4. The structure of the dictyosome of the Golgi complex ([https://en.wikipedia.org/wiki/Golgi_apparatus#/media/File:Golgi_apparatus_\(borderless_version\)-en.svg](https://en.wikipedia.org/wiki/Golgi_apparatus#/media/File:Golgi_apparatus_(borderless_version)-en.svg))

The vesicles that leave the rough endoplasmic reticulum are transported to the cis face of the Golgi apparatus, where they fuse with the Golgi membrane and empty their contents into the lumen. Once inside the lumen, the molecules are modified, then sorted for transport to their next destinations.

Those proteins destined for areas of the cell other than either the endoplasmic reticulum or the Golgi apparatus are moved through the Golgi cisternae towards the trans face, to a complex network of membranes and associated vesicles known

as the trans-Golgi network (TGN). This area of the Golgi is the point at which proteins are sorted and shipped to their intended destinations by their placement into one of at least three different types of vesicles, depending upon the signal sequence they carry (**fig. 6.5**).

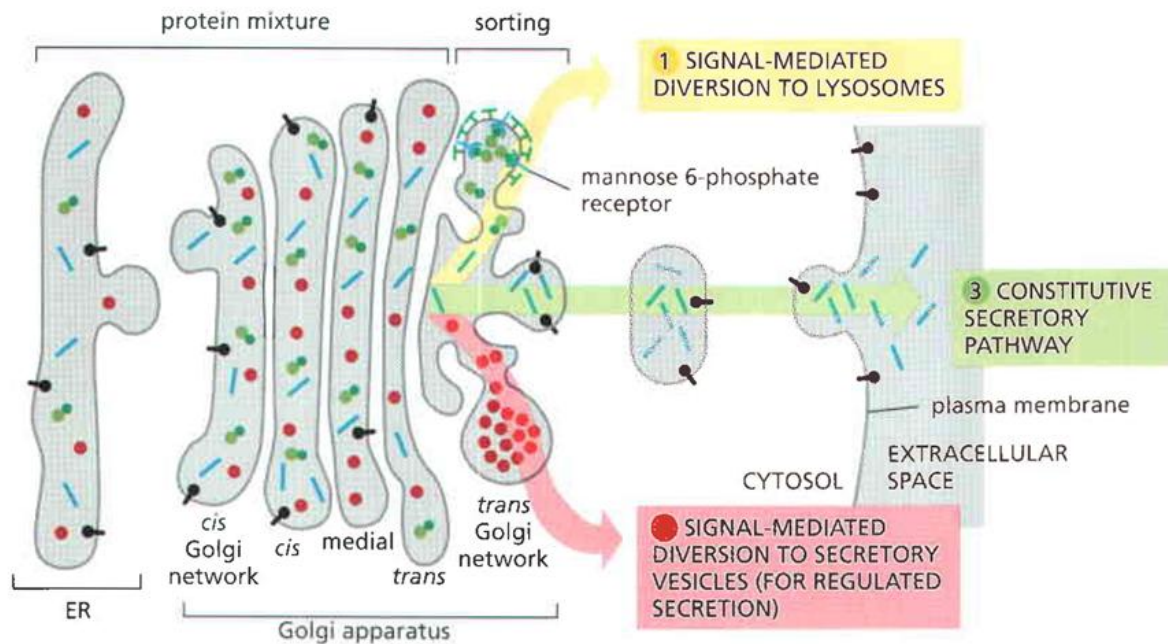


Figure 6.5. The three best-understood pathways of protein sorting in the trans Golgi network (Alberts et al., 2008, p. 801)

(1) Proteins with the mannose 6-phosphate (M6P) marker are diverted to lysosomes (via endosomes) in clathrin-coated transport vesicles. (2) Proteins with signals directing them to secretory vesicles are concentrated in such vesicles as part of a regulated secretory pathway that is present only in specialized secretory cells. (3) In unpolarized cells, a constitutive secretory pathway delivers proteins with no special features to the cell surface. In polarized cells, such as epithelial cells, however, secreted and plasma membrane proteins are selectively directed to either the apical or the basolateral plasma membrane domain, so a specific signal must mediate at least one of these two pathways, as we discuss later

In the cis and medial zones of dictyosomes all these proteins move together, while their modification occurs separately, depending on their oligosaccharide markers. The actual separation of proteins and their sorting takes place in the trans-pole of the Golgi complex, by means of a special mechanism for spatial separation of different proteins and their pathways. The first flow is constituted by hydrolytic enzymes directed to the lysosome compartment. Trans-pole membranes contain a transmembrane receptor protein (mannose-6-phosphate receptor) that recognizes and binds to phosphorylated mannose groups in the oligosaccharide chain of lysosomal enzymes.

The binding occurs at neutral pH values inside the trans-pole cisternae. Next, clathrine-coated primary lysosomes bud off of the membranes. Subsequently, the primary lysosomes lose their clathrine, merge with endosomes, and secondary

lysosomes (digestive vacuoles) are formed. Due to the activity of the proton carrier, acidification of the medium occurs inside these lysosomes. As pH falls below 6, lysosomal enzymes dissociate from the M-6-P receptors, they are activated and begin to digest organic compounds. The membrane sections together with the M-6-P-receptors are returned to the membrane of the trans Golgi network by recycling the membrane vesicles. The formation of immature (primary) lysosomes, their fusion with endosomes and the formation of secondary lysosomes reflect the essence of the heterophagic cycle or the digestion cycle in the cell (**fig. 6.6**).

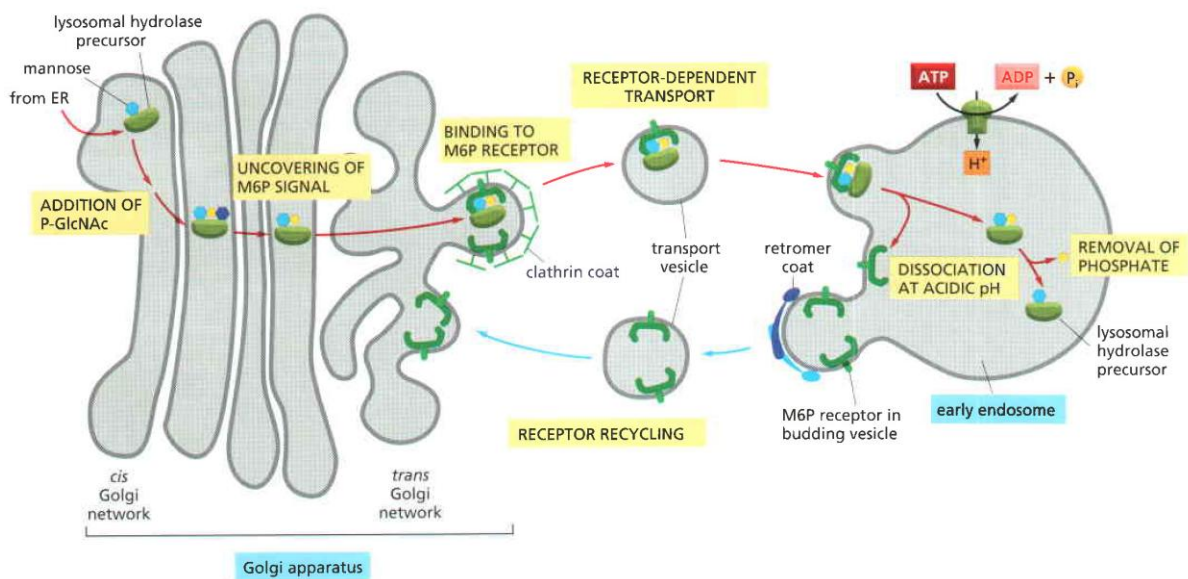


Figure 6.6. The transport of newly synthesized lysosomal hydrolases to lysosomes (Alberts et al., 2008, p. 785)

The sequential action of two enzymes in the cis and trans Golgi network adds mannose 6-phosphate (M6P) groups to the precursors of lysosomal enzymes. They then segregate from all other types of proteins in the TGN because monomeric adaptor proteins in the clathrin coat bind the M6P receptors, which, in turn, bind the modified lysosomal hydrolases. The clathrin-coated vesicles bud off from the TGN, shed their coat, and fuse with early endosomes. At the lower pH of the endosome, the hydrolases dissociate from the M6P receptors, and the empty receptors are recycled in retromer-coated vesicles to the Golgi apparatus for further rounds of transport. In the endosomes, the phosphate is removed from the mannose sugars attached to the hydrolases, further ensuring that the hydrolases do not return to the Golgi apparatus with the receptor

Second stream (exocytotic vesicles, constitutive): vesicle contains proteins destined for extracellular release. After packaging, the vesicles bud off and immediately move towards the plasma membrane, where they fuse and release the contents into the extracellular space in a process known as constitutive secretion.

Third stream (secretory vesicles, regulated): vesicles contain proteins destined for extracellular release. After packaging, the vesicles bud off and are stored in the cell until a signal is given for their release. When the appropriate signal is received, they move toward the membrane and fuse to release their contents. This process is known as regulated secretion.

6.3. Lysosomes

Lysosomes are membrane-enclosed compartments filled with soluble hydrolytic enzymes that control intracellular digestion of macromolecules.

Lysosomes contain about 40 types of hydrolytic enzymes, including proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases. All are acid hydrolases. For optimal activity, they need to be activated by proteolytic cleavage and require an acid environment, which the lysosome provides by maintaining a pH of about 4.5–5.0 in its interior (**fig. 6.7**). By this arrangement, the contents of the cytosol are doubly protected against attack by the cell's own digestive system: the membrane of the lysosome keeps the digestive enzymes out of the cytosol, but even if they leak out, they can do little damage at the cytosolic pH of about 7.2.

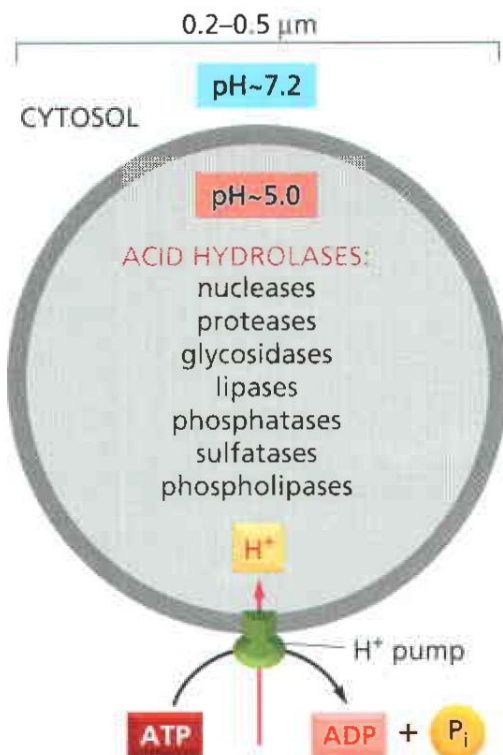


Figure 6.7. Lysosomes (Alberts et al., 2008, p. 780)

The acid hydrolases are hydrolytic enzymes that are active under acidic conditions. ATPase in the membrane pumps H⁺ into the lysosome, maintaining its lumen at an acidic pH

Like all other intracellular organelles, the lysosome not only contains a unique collection of enzymes, but also has a unique surrounding membrane. Most of the lysosomal membrane proteins, for example, are unusually highly glycosylated, which helps to protect them from the lysosomal proteases in the lumen. Transport proteins in the lysosomal membrane carry the final products of the digestion of macromolecules – such as amino acids, sugars, and nucleotides – to the cytosol, where the cell can either reuse or excrete them.

A vacuolar H⁺ ATPase in the lysosomal membrane uses the energy of ATP hydrolysis to pump H⁺ into the lysosome, thereby maintaining the lumen at its acidic pH. In addition to providing a low-pH environment that is suitable for

reactions occurring in the organelle lumen, the H^+ gradient provides a source of energy that drives the transport of small metabolites across the organelle membrane.

6.4. Peroxisomes

All eukaryotic cells have peroxisomes. They contain oxidative enzymes, such as catalase and urate oxidase, at such high concentrations that, in some cells, the peroxisomes stand because of the presence of a crystalloid core (**fig. 6.8**).

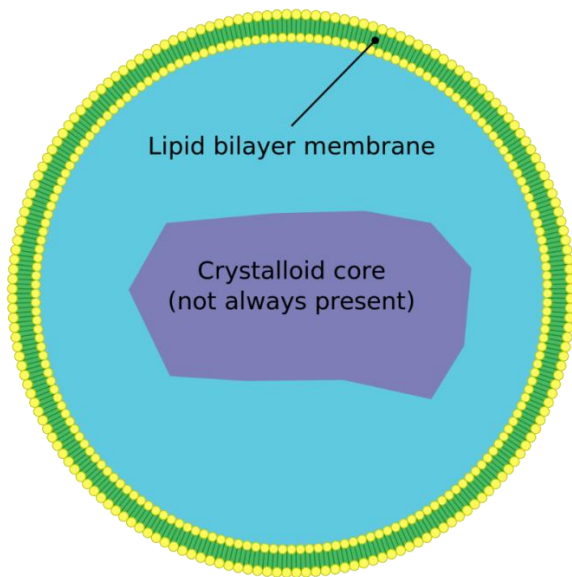
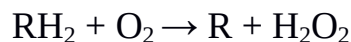
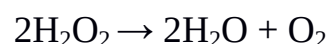


Figure 6.8. The structure of a peroxisome
<https://commons.wikimedia.org/wiki/File:Peroxisome.svg>

Peroxisomes are so named because they usually contain one or more enzymes that use molecular oxygen to remove hydrogen atoms from specific organic substrates (designated here as R) in an oxidation reaction that produces hydrogen peroxide (H_2O_2):



Catalase uses the H_2O_2 generated by other enzymes in the organelle to oxidize a variety of other substrates – including phenols, formic acid, formaldehyde, and alcohol – by the «peroxidation» reaction: $H_2O_2 + R'H_2 \rightarrow R' + 2H_2O$. This type of oxidation reaction is particularly important in liver and kidney cells, where the peroxisomes detoxify various toxic molecules that enter the bloodstream. About 25% of the ethanol we drink is oxidized to acetaldehyde in this way. In addition, when excess $H_2O_2 \rightarrow$ accumulates in the cell, catalase converts it to H_2O through the reaction:



A major function of the oxidation reactions performed in peroxisomes is the breakdown of fatty acid molecules. The process called β -oxidation shortens the

alkyl chains of fatty acids sequentially in blocks of two carbon atoms at a time, thereby converting the fatty acids to acetyl CoA. The peroxisomes then export the acetyl CoA to the cytosol for reuse in biosynthetic reactions. In mammalian cells, beta-oxidation occurs in both mitochondria and peroxisomes; in yeast and plant cells, however, this essential reaction occurs exclusively in peroxisomes.

Peroxisomes are unusually diverse organelles, and even in the various cell types of a single organism they may contain different sets of enzymes. They also adapt remarkably to changing conditions.

It has long been debated whether new peroxisomes arise from preexisting ones by organelle growth and fission – and therefore replicate in an autonomous way as mentioned earlier for mitochondria and plastids – or whether they derive as a specialized compartment from the endoplasmic reticulum (ER). Aspects of both views may be true (**fig. 6.9**). Most peroxisomal membrane proteins are made in the cytosol and insert into the membrane of preexisting ones, yet others are first integrated into the ER membrane from where they may bud in specialized peroxisomal precursor vesicles. New precursor vesicles may then fuse with one another and begin importing additional peroxisomal proteins using their own protein import machinery to grow into mature peroxisomes, which can enter into a cycle of growth and fission.

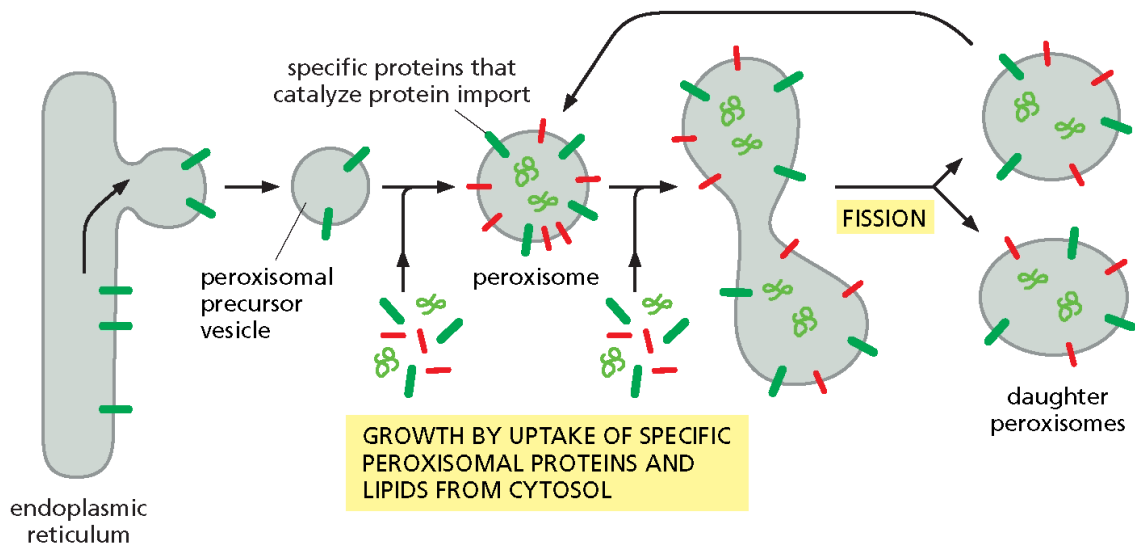


Figure 6.9. A model that explains how peroxisomes proliferate and how new peroxisomes may arise (Alberts et al., 2008, p. 757)

Peroxisome precursor vesicles are thought to bud from the ER. The machinery that drives the budding reaction and that selects only peroxisomal proteins for packaging into these vesicles, and not ER proteins or proteins destined for other locations in the cell, is not known. Peroxisome precursor vesicles may then fuse with one another or with preexisting peroxisomes. The peroxisome membrane contains import receptor proteins. Cytosolic ribosomes synthesize peroxisomal proteins, including new copies of the import receptor, and then import them into the organelle. Presumably, the lipids required for growth are also imported, although some may derive directly from the ER in the membrane of peroxisome precursor vesicles

CONTROL TESTS

Read attentively each assignment (question) and choose one correct answer from the choices offered below.

1. The vacuolar system includes:

- a) single-membrane organoids: endoplasmic reticulum, Golgi complex, lysosomes, peroxysomes, secretory vacuoles;
- b) two-membrane organoids: endoplasmic reticulum, Golgi complex, lysosomes, peroxysomes, secretory vacuoles;
- c) single-membrane organoids: endoplasmic reticulum and two-membrane organoids: Golgi complex, lysosomes, peroxysomes.

2. The function of the vacuolar system is:

- a) synthesis, modification, sorting and export of biopolymers from a cell;
- b) synthesis, modification, reduplication and excretion (export) of biopolymers from a cell;
- c) synthesis, modification and excretion (export) of biopolymers from a cell.

3. Ribosomes are attached by a SRP particle to ER membranes by means of:

- a) integral proteins – ribophorins;
- b) m-RNA;
- c) phospholipids.

4. What is a translocon?

- a) a protein complex located on an ER membrane;
- b) a ribosome section attached to an ER membrane;
- c) a growing polypeptide chain.

TOPICS FOR INDEPENDENT WORK

Independent work includes the study of the following topics:

Topic 1: Lysosomes. Structure of lysosomes and their chemical characteristics.

Topic 2: Relationship between rough ER, the nuclear envelope and the Golgi complex.

Topic 3: Peroxysomes (microbodies). Peroxisome structure and chemical characteristics. Functions of peroxisomes.

Topic 4: Heterophagic and autophagic cycles in a cell. «Accumulation» diseases.

Topic 5: Structural and functional interrelation of all compartments of the vacuolar system.

MITOCHONDRIA

7.1. Structure and function of mitochondria

The mitochondrion is a double-membrane-bound organelle found in most eukaryotic organisms. Some cells in some multicellular organisms may, however, lack them (for example, mature mammalian red blood cells). There are two hypotheses about the origin of mitochondria: endosymbiotic and autogenous. The endosymbiotic hypothesis suggests that mitochondria were originally prokaryotic cells, capable of implementing oxidative mechanisms that were not possible for eukaryotic cells; they became endosymbionts living inside the eukaryote. In the autogenous hypothesis, mitochondria were born by splitting off a portion of DNA from the nucleus of the eukaryotic cell at the time of divergence with the prokaryotes; this DNA portion would have been enclosed by membranes, which could not be crossed by proteins. Since mitochondria have many features in common with bacteria, the endosymbiotic hypothesis is more widely accepted.

Mitochondria are commonly between 0.75 and 3 μm^2 in area but vary considerably in size and structure. The number of mitochondria in a cell can vary widely by organism, tissue, and cell type. For instance, red blood cells have no mitochondria, whereas liver cells can have more than 2000. The organelle is composed of compartments that carry out specialized functions. These compartments or regions include the outer membrane, the intermembrane space, the inner membrane, and the cristae and matrix (**fig. 7.1**). Mitochondria generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy. A mitochondrion is thus termed the powerhouse of the cell.

Although most of a cell's DNA is contained in the cell nucleus, the mitochondrion has its own independent genome that shows substantial similarity to bacterial genomes. Mitochondrial proteins (proteins transcribed from mitochondrial DNA) vary depending on the tissue and the species.

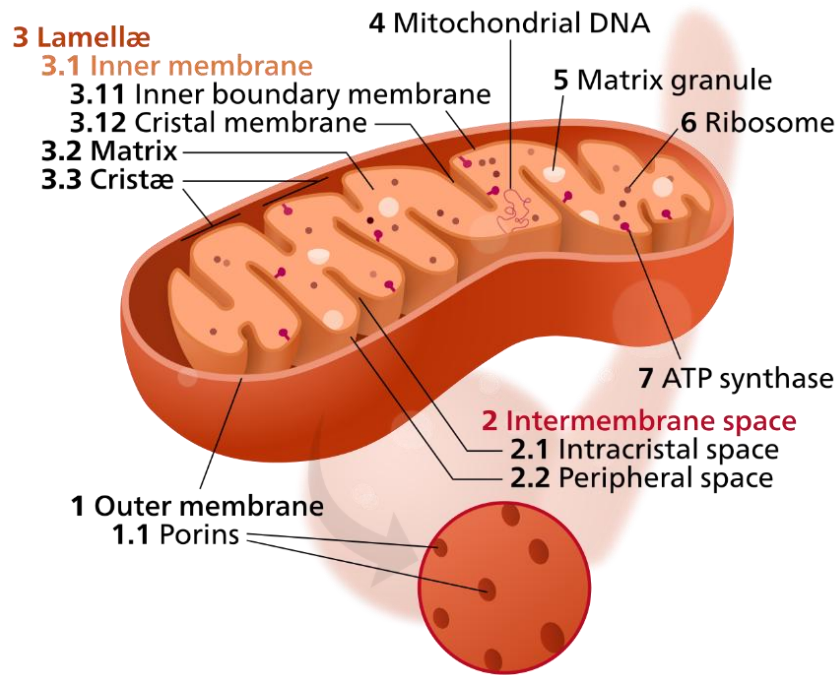


Figure 7.1. Components of a typical mitochondrion (<https://en.wikipedia.org/wiki/Mitochondrion>)

1 – outer membrane; 1.1 – porin; 2 – intermembrane space: 2.1 – intracristal space; 2.2 – peripheral space; 3 – lamella; 3.1 – inner membrane; 3.11 – inner boundary membrane; 3.12 – cristal membrane; 3.2 – matrix; 3.3 – cristæ; 4 – mitochondrial DNA; 5 – matrix granule; 6 – ribosome; 7 – ATP synthase

7.2. Localization of the main links of oxidative phosphorylation in the membranes of mitochondria

Unlike the outer membrane, the inner membrane contains large amounts of proteins (up to 75%). These proteins can be roughly divided into three groups: respiratory chain enzymes (electron transfer chain proteins); ATP synthesis enzymes (ATP synthases); transport proteins.

The respiratory chain is the main system for converting energy in mitochondria. Here, the enzymes of the respiratory chain are gradually oxidized and reduced, resulting in the release of energy in small portions. The respiratory chain is a series of protein complexes embedded in the inner mitochondrial membrane. It contains three main enzyme complexes. The first one, the NADH-dehydrogenase complex (1) receives electrons and protons from the nicotinamide adenine dinucleotide (NADH)-transmitters and takes them to the ubiquinone (4), and then to the second complex, the *b-c* complex (2), which in its turn receives only electrons, and protons are discharged into the intermembrane space (proton reservoir). Electrons are transferred to the cytochrome *c* (5) and then to the third cytochrome oxidase complex (3), which provides the transfer of protons into the intermembrane space, while electrons are transferred to oxygen (**fig. 7.2**).

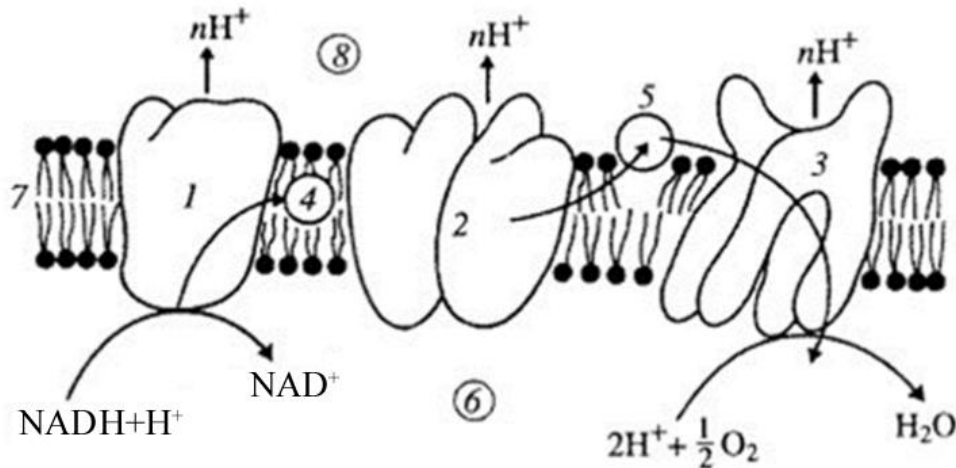


Figure 7.2. Electron transport chain in the mitochondrial intermembrane space (Chentsov, 2015, p. 337)

1 – NADH-dehydrogenase complex; 2 – *b-c* complex; 3 – cytochrome oxidase complex; 4 – ubiquinone; 5 – cytochrome *c*; 6 – mitochondrial matrix; 7 – internal mitochondrial membrane; 8 – inter-membrane space

Iron-containing pigments – cytochromes are the most important enzymes in the respiratory chain. They differ from each other in the degree of affinity to electrons, which is expressed through the redox potential: the higher this potential, the greater the affinity of the oxidized molecule to electrons. The electrons pass through the chain along the potential gradient – from cytochrome *b* to cytochrome *c*, then to the cytochrome complex *a*, and finally to oxygen. This reaction, whereby oxygen is activated, is considered the main stage of oxidative metabolism.

ATP synthases, large mushroom-shaped protein complexes (8–9 nm in diameter) consisting of 9 subunits, are embedded into the inner membrane of the mitochondria. They consist of two main parts: the coupling factor (a mushroom-like headpiece, the Racker factor) and the membrane component, a proton-conducting channel through which protons from the intermembrane space flow back to the mitochondrial matrix over an electrochemical gradient. The membrane component of the ATP synthase is embedded into the inner membrane of the mitochondria, and the head faces the matrix (**fig. 7.3**).

The redox energy from NADH and FADH_2 is transferred to oxygen (O_2) in several steps via the electron transport chain. These energy-rich molecules are produced within the matrix via the citric acid cycle but are also produced in the cytoplasm by glycolysis. Reducing equivalents from the cytoplasm can be imported via the malate-aspartate shuttle system of antiporter proteins or feed into the electron transport chain using a glycerol phosphate shuttle. Protein complexes in the inner membrane: NADH–dehydrogenase (ubiquinone), cytochrome *c* reductase, and cytochrome *c* oxidase) perform the transfer and the incremental release of energy is used to pump protons (H^+) into the intermembrane space.

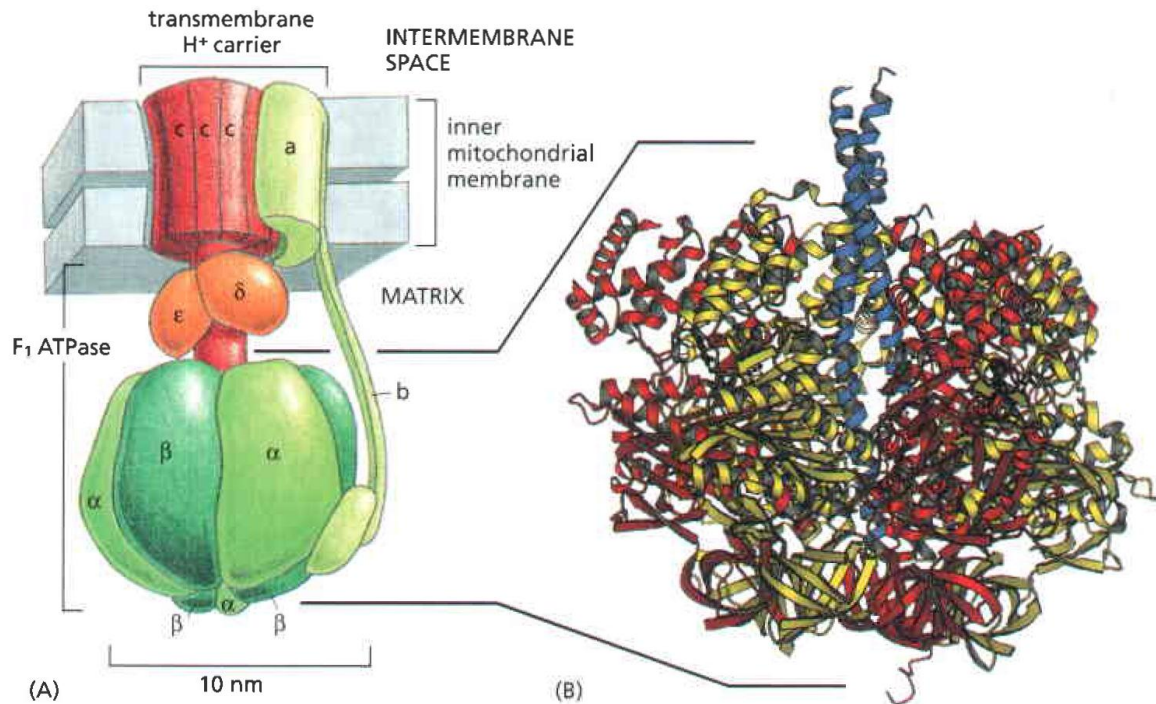


Figure 7.3. ATP synthase (Alberts et al., 2008, p. 822)

The enzyme is composed of a head portion, called the F_1 ATPase, and a transmembrane H^+ carrier, called F_0 . Both F_1 and F_0 are formed from multiple subunits, as indicated. A rotating stalk is fixed to a rotor (red) formed by a ring of 10–14 c subunits in the membrane. The stator (green) is formed from transmembrane a subunit, tied to other subunits that create an elongated arm. This arm fixes the stator to a ring of 3 α and 3 β subunits that forms the head, which likewise cannot rotate. (B) The three-dimensional structure of the F_1 ATPase, determined by x-ray crystallography. This part of the ATP synthase derives its name from its ability to carry out the reverse of the ATP synthesis reaction – namely, the hydrolysis of ATP to ADP and P_{if} when detached from the transmembrane portion. (B, courtesy of John Walker, from J.P. Abrahams et al., *Nature* 370:621-628, 1994. With permission from Macmillan Publishers Ltd.)

7.3. Mechanism of oxidative phosphorylation

The transport of protons and electrons through the enzymes of the respiratory chain results in the accumulation of positively charged protons in the intermembrane space. Due to this, the outer side of the inner membrane is positively charged and electrons are transferred to the inner surface of the inner membrane, where they eventually combine with oxygen and the inner side of the membrane becomes negatively charged. When a certain potential difference (200 mV) is reached, the positively charged protons are pushed through the ATP synthase channel by the electric field back into the matrix, and the energy of the moving protons is used in the phosphorylation reaction of ADP to ATP (**fig. 7.4**). Thus, ATP synthesis is coupled with the reverse flow of protons from the intermembrane space into the mitochondrial matrix. ATP synthase converts motion

energy into chemical bond energy by synthesizing ATP from ADP and inorganic phosphate. The proton gradient through the inner membrane of the mitochondria gives 24 kJ of energy per mole of protons transferred. One ATP molecule is formed by the passage of 2 protons. The action of ATP synthase is reversible. It can use both the ATP hydrolysis energy to pump protons through the membrane and the proton flow energy for ATP synthesis. In other words, ATP synthase is a reversible conjugating system that accomplishes the interconversion of the energy of the electrochemical proton gradient and of chemical bonds.

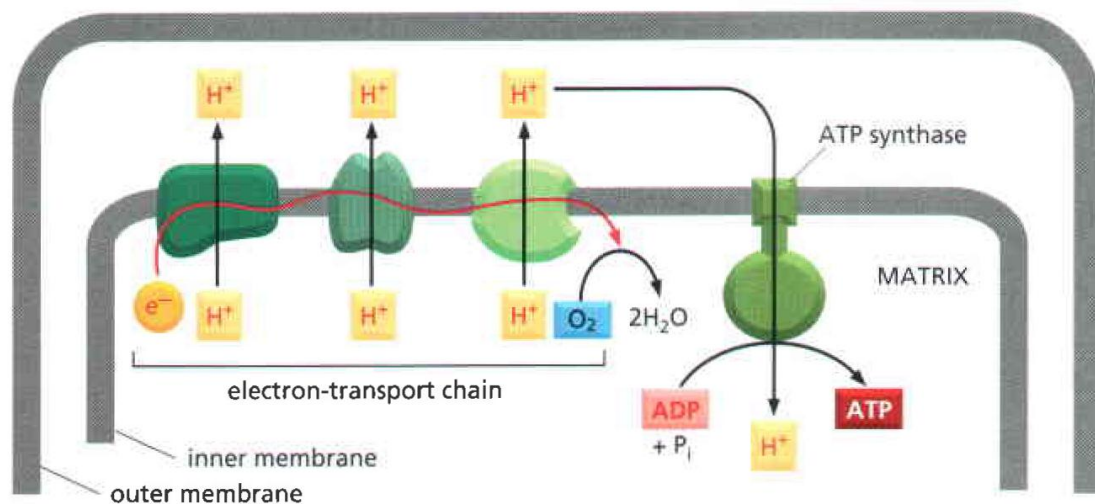


Figure 7.4. The general mechanism of oxidative phosphorylation (Alberts et al., 2008, p. 821)

As a high-energy electron is passed along the electron-transport chain, some of the energy released drives the three respiratory enzyme complexes that pump H^+ out of the matrix. The resulting electrochemical proton gradient across the inner membrane drives H^+ back through the ATP synthase, a transmembrane protein complex that uses the energy of the H^+ flow to synthesize ATP from ADP and P_i in the matrix

In mitochondria, the electron transfer process (oxidation) is coupled with phosphorylation; the whole process is called oxidative phosphorylation or chemiosmotic mechanism. The chemiosmotic theory explaining the mechanism of energy transformation in a biological membrane during the synthesis of adenositriphosphate (ATP) was developed by the English biochemist Peter Mitchell (1978 Nobel Prize). The chemiosmotic hypothesis refers to the stage of oxidative phosphorylation, which begins with electron transport in the respiratory chain and ends with the synthesis of ATP molecules. The main point of Mitchell's hypothesis comes down to the statement that as each pair of electrons passes through the enzymes of the respiratory chain from the carrier molecules ($NAD \cdot H$) to oxygen through the inner membrane of the mitochondria, six protons are translocated from the matrix to the intermembrane space, and six OH^- ions remain inside the matrix. Protons are transferred in three cycles in such a way that a pair of electrons crosses the membrane in both directions three times. During each cycle, two protons are transferred from the matrix to the proton reservoir.

CONTROL TESTS

Read attentively each assignment (question) and choose one correct answer from the choices offered below.

1. What are cristae?

- a) the folds of the inner mitochondrial membrane;
- b) the folds of the outer mitochondrial membrane;
- c) inter-membrane formations.

2. What is the main function of mitochondria?

- a) conversion of energy from organic compounds into ATP energy;
- b) conversion of ATP energy into energy of organic compounds;
- c) synthesis of energy-rich fat-like substances.

3. Finish the sentence: metabolism is a set of reactions of....

- a) assimilation and dissimilation;
- b) dissimilation and catabolism;
- c) anabolism and assimilation.

4. What is a respiratory chain or an electron transport chain?

- a) a system of protein complexes embedded in the inner mitochondrial membrane that carries protons and electrons;
- b) a system of proton carriers;
- c) a system of protein complexes embedded in the inner mitochondrial membrane that carries electrons.

TOPICS FOR INDEPENDENT WORK

Independent work includes the study of the following topics:

Topic 1: Energy metabolism. Oxidation of organic substances. Stages of energy metabolism: preparatory, anaerobic glycolysis, cellular respiration.

Topic 2: Mitochondrial apparatus (chondriome). Morphology, localization and structure of mitochondria. Composition and properties of external and internal mitochondrial membranes.

Topic 3: Mitochondrion as a semi-autonomous organoid. Mitochondrial matrix: RNA, ribosomes, DNA and mitochondrial proteins.

Topic 4: Proton-motive force mechanism in thylakoid membranes of chloroplasts.

THE CENTROSOME

8.1. Centrioles

In cell biology, a centriole is a cylindrical organelle composed mainly of a protein called tubulin. Centrioles are not present in all eukaryotes; for example, they are absent from conifers (Pinophyta), flowering plants (Angiosperms) and most fungi, and are only present in the male gametes of charophytes, bryophytes, seedless vascular plants, cycads, and ginkgo. In fungi and diatoms, microtubules are nucleated at an MTOC that is embedded in the nuclear envelope as a small plaque called the spindle pole body. Higher-plant cells seem to nucleate microtubules at sites distributed all around the nuclear envelope. Neither fungi nor most plant cells contain centrioles. Despite these differences, all these cells contain γ -tubulin and seem to use it to nucleate their microtubules.

In animal cells, the astral configuration of microtubules is very robust, with dynamic plus ends pointing outward toward the cell periphery and stable minus ends collected near the nucleus.

Centrioles are typically made up of nine sets of short microtubule triplets, arranged in a cylinder, plus a large number of accessory proteins. Centrioles are involved in the organization of the mitotic spindle and in the completion of cytokinesis. A bound pair of centrioles, surrounded by a shapeless mass of dense material, called the pericentriolar material (PCM), makes up a structure called a centrosome (*fig. 8.1*).

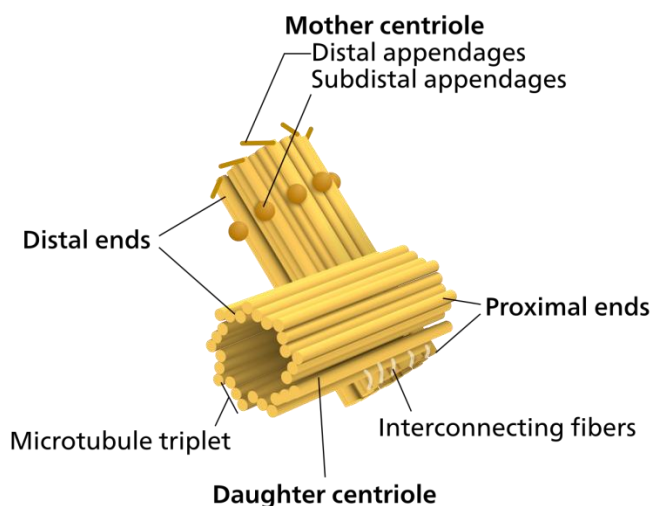


Figure 8.1. A mother and daughter centriole, attached orthogonally
<https://en.wikipedia.org/wiki/Centriole>

Embedded in the centrosome is a pair of somewhat mysterious cylindrical structures arranged at right angles to each other in an L-shaped configuration. The centrioles organize the centrosome matrix (also called the pericentriolar material), ensuring its duplication during each cell cycle as the centrioles themselves duplicate. Each centrosome contains a pair of centrioles; although the centrioles have duplicated, they remain together in a single complex. The two centrioles have partly separated during the isolation procedure but remain tethered together by fine fibers, which keep the centriole pair together until it is time for them to separate. Both centrioles are cut longitudinally, and it can now be seen that the two have different structures: the mother centriole is larger and more complex than the daughter centriole, and only the mother centriole is associated with pericentriolar matrix that nucleates microtubules (**fig. 8.2**).

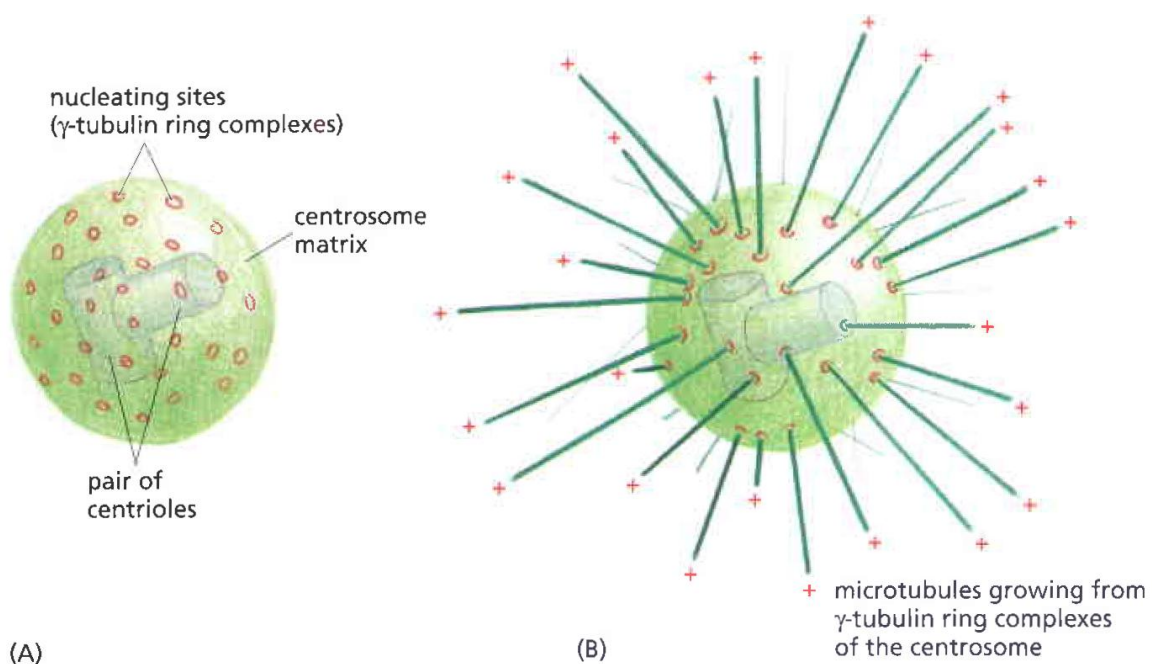


Figure 8.2. The centrosome (Alberts et al., 2008, p. 993)

(A) The centrosome is the major MTOC of animal cells. Located in the cytoplasm next to the nucleus, it consists of an amorphous matrix of fibrous proteins to which the γ -tubulin ring complexes that nucleate microtubule growth are attached. This matrix is organized by a pair of centrioles, as described in the text. (B) A centrosome with attached microtubules. The minus end of each microtubule is embedded in the centrosome, having grown from a γ -tubulin ring complex, whereas the plus end of each microtubule is free in the cytoplasm

8.2. Centriole replication

The centrosome duplicates and splits into two equal parts during interphase, each half containing a duplicated centriole pair (**fig. 8.3**). These two daughter centrosomes move to opposite sides of the nucleus when mitosis begins, and they form the two poles of the mitotic spindle.

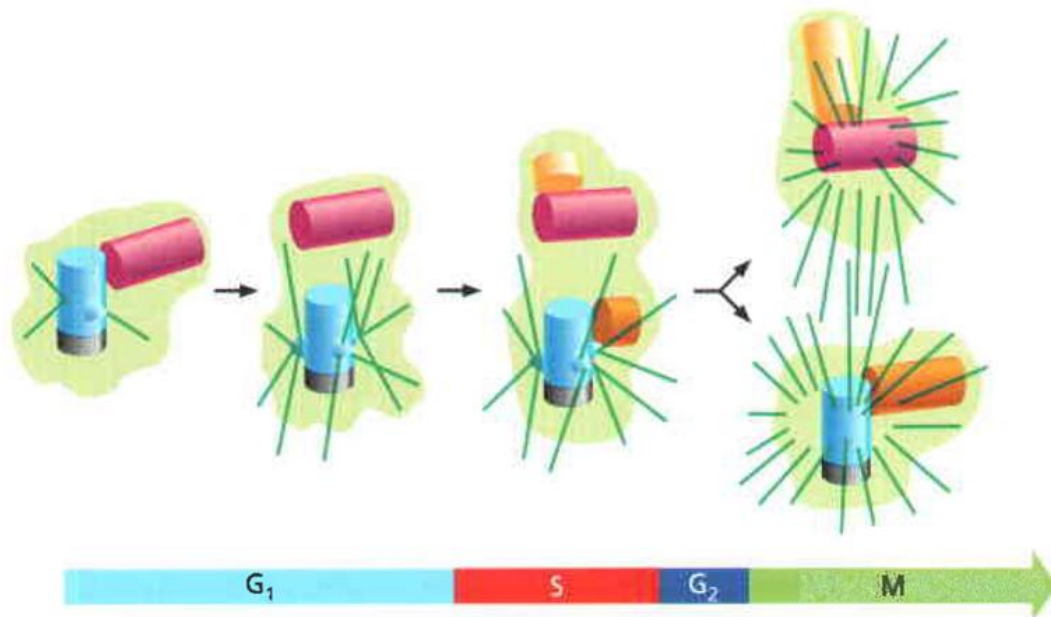


Figure 8.3. Centriole replication (Alberts et al., 2008, p. 1072–1073)

The centrosome consists of a centriole pair and associated pericentriolar matrix (*green*). At a certain point in G₁, the two centrioles of the pair separate by a few micrometers. During S phase, a daughter centriole begins to grow near the base of each mother centriole and at a right angle to it. The elongation of the daughter centriole is usually completed by G₂. The two centriole pairs remain close together in a single centrosomal complex until the beginning of M phase, when the complex splits in two and the two halves begin to separate. Each centrosome now nucleates its own radial array of microtubules called an aster

The core of the mitotic spindle is a bipolar array of microtubules, the minus ends of which are focused at the two spindle poles, and the plus ends of which radiate outward from the poles (**fig. 8.4**).

The plus ends of some microtubules, called the interpolar microtubules, interact with the plus ends of microtubules from the other pole, resulting in an antiparallel array in the spindle midzone. The plus ends of other microtubules, the kinetochore microtubules, are attached to sister chromatid pairs at large protein structures called kinetochores, which are located at the centromere of each sister chromatid. Finally, many spindles also contain astral microtubules that radiate outward from the poles and contact the cell cortex, helping to position the spindle in the cell. In most somatic animal cells, each spindle pole is focused at a protein organelle the centrosome. The pericentriolar matrix nucleates a radial array of microtubules, with their fast-growing plus ends projecting outward and their minus ends associated with the centrosome. The matrix contains a variety of proteins, including microtubule-dependent motor proteins.

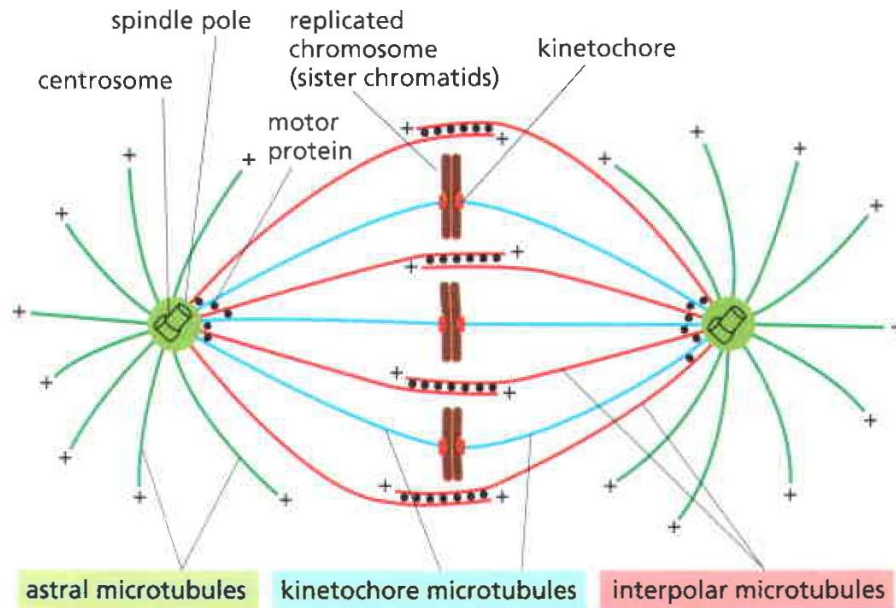


Figure 8.4. The three classes of microtubules of the mitotic spindle in an animal cell (Alberts et al., 2008, p. 1076)

The plus ends of the microtubules project away from the centrosomes, while the minus ends are anchored at the spindle poles, which in this example are organized by centrosomes. Kinetochore microtubules connect the spindle poles with the kinetochores of sister chromatids, while interpolar microtubules from the two poles interdigitate at the spindle equator. Astral microtubules radiate out from the poles into the cytoplasm and usually interact with the cell cortex, helping to position the spindle in the cell

CONTROL TESTS

Read attentively each assignment (question) and choose one correct answer from the choices offered below.

1. The main structural protein of centrioles is:

- a) keratin;
- b) actin;
- c) tubulin;
- d) myosin.

2. The structural formula = $(9 \cdot 3) + 0$ describes the structure of:

- a) centriole;
- b) axoneme;
- c) basal body;
- d) flagellum.

3. Centrosomes are a system of organized:

- a) microfilaments;
- b) microtubules;

- c) myofibrils;
- d) neurofibrils.

4. In animal cells, two centrioles located near the nucleus form...

- a) a dictyosome;
- b) a diplotene;
- c) a diplosome;
- d) a bivalent.

TOPICS FOR INDEPENDENT WORK

Independent work includes the study of the following topics:

Topic 1: Structure of mother and daughter centrioles, diplosomes and centrosomes in animal eukaryotic cells.

Topic 2: Functional activity of the mother centriole as a center for cytoskeleton microtubules formation.

Topic 3: Basal bodies. Formation of primary cilia.

EUKARYOTIC CELL NUCLEUS

The nucleus is the most important component of eukaryotic cells. It is not only a reservoir of genetic material, but also the place where this material is reproduced and functions (**fig. 9.1**).

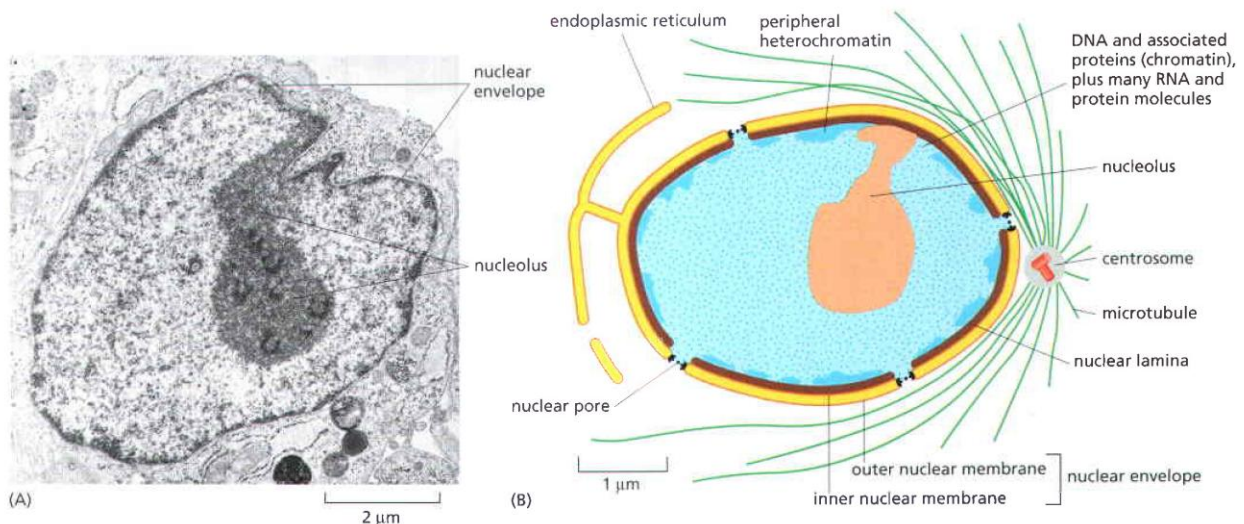


Figure 9.1. A cross-sectional view of a typical cell nucleus (Alberts et al., 2008, p. 201)

(A) Electron micrograph of a thin section through the nucleus of a human fibroblast. (B) Schematic drawing, showing that the nuclear envelope consists of two membranes, the outer one being continuous with the endoplasmic reticulum membrane. The space inside the endoplasmic reticulum (the ER lumen) is colored yellow; it is continuous with the space between the two nuclear membranes. The lipid bilayers of the inner and outer nuclear membranes are connected at each nuclear pore. A sheet-like network of intermediate filaments (brown) inside the nucleus provides mechanical support for the nuclear envelope, forming a special supporting structure called the nuclear lamina. The heterochromatin near the lamina contains specially condensed regions of DNA

The main elements of the eukaryotic nucleus structure in an animal cell are:

1. Chromatin that performs a genetic function.
2. The nucleus surface apparatus that performs barrier, receptor, transport and scaffolding functions.
3. The nucleolus where rRNA synthesis takes place and where ribosomal sub-particles are formed.
4. The nuclear protein matrix that provides spatial arrangement of chromosomes in the nucleus.
5. The karyoplasm (nucleoplasm or nucleus sap) is a liquid phase of the cell nucleus in which the processes of nuclear metabolism and transport take place.

9.1. Chromatin

The term chromatin was suggested by the German scientist Walther Flemming in 1880. Chromatin is a complex of DNA (40%) and protein (60%) found in eukaryotic cells. The proteins that bind to the DNA to form eukaryotic chromosomes are traditionally divided into two general classes: the histones and the non-histone chromosomal proteins. The complex of both classes of protein with the nuclear DNA of eukaryotic cells is known as chromatin. Histones are present in such enormous quantities in the cell (about 60 million molecules of each type per human cell) that their total mass in chromatin is about equal to that of the DNA. Besides, chromatin also contains a small amount of RNA.

In the interphase nucleus, chromatin is represented by alternating dense blocks (heterochromatin), and areas of loose euchromatin, which results in a peculiar structure of the interphase nucleus. Heterochromatin, in turn, is divided into constitutive (permanent) and facultative heterochromatin. The constitutive heterochromatin is not genetically active, it is not transcribed, it is replicated later than the rest of the chromatin. It contains a special (satellite) DNA and it acts as a spacer between the genes. The facultative heterochromatin can change the degree of its condensation and can turn to the state of euchromatin.

The density of chromatin packaging is determined by the basic proteins, histones.

9.2. Histones, basic chromatin proteins

Histones are basic proteins with a high content of amino acids (e.g., lysine and arginine). Positive charges on amino acid groups cause the salt or electrostatic coupling of these proteins with negative charges on DNA phosphate groups. This coupling is quite unstable and easily falls apart. The histones are divided into five groups: H1, H2A, H2B, H3 and H4. The histone classes differ from each other in the content of different basic amino acids. Histone H1 is not a unique molecule, but a class of closely related proteins, and their content in chromatin is half that of other classes.

Histones of all classes are characterized by cluster distribution of basic amino acids at N- and C-termini of molecules. The flexible N-terminus can be modified by methylation reaction, i.e. by introduction of a methyl group (CH_3) into the protein instead of a hydrogen atom, or by acetylation, i.e. introduction of acetic acid residue (CH_3CO), or phosphorylation, i.e. introduction of phosphoric acid residue (PO_4). Acetylation and methylation result in the loss of positive charges and phosphorylation introduces a negative charge. These modifications are reversible and significantly change the ability of histones to interact with DNA. Acetylation is necessary to form a less compact chromatin structure, i.e. it is associated with gene activation, while phosphorylation and methylation leads to condensation of chromatin and, accordingly, to inactivation of genes.

Consequently, histone proteins regulate the level of transcription. The quantity and quality of histones affect the compactness and activity of chromatin.

9.3. Packaging of DNA molecules

The DNA packaging process involves several levels: 1. Nucleosomal; 2. Nucleomeric (30-nm fibrils); 3. Chromomeric (loop domains); 4. Chromonemic.

Histones are responsible for the first and most basic level of chromosome packing, the nucleosome, a protein-DNA complex discovered in 1974. When interphase nuclei are broken open very gently and their contents examined under the electron microscope, most of the chromatin is in the form of a fiber with a diameter of about 30 nm. If this chromatin is subjected to treatments that cause it to unfold partially, it can be seen under the electron microscope as a series of «beads on a string». The string is DNA, and each bead is a «nucleosome core particle» that consists of DNA wound around a protein core formed from histones.

The structural organization of nucleosomes was determined after first isolating them from unfolded chromatin by digestion with particular enzymes (called nucleases) that break down DNA by cutting between the nucleosomes. After digestion for a short period, the exposed DNA between the nucleosome core particles, the linker DNA, is degraded. Each individual nucleosome core particle consists of a complex of eight histone proteins – two molecules each of histones H2A, H2B, H3, and H4 – and double-stranded DNA that is 147 nucleotide pairs long. The histone octamer forms a protein core around which the double-stranded DNA is wound (*fig. 9.2*).

Each nucleosome core particle is separated from the next by a region of linker DNA, which can vary in length from a few nucleotides pairs up to about 80. (The term nucleosome technically refers to a nucleosome core particle plus one of its adjacent DNA linkers, but it is often used synonymously with nucleosome core particle.) On average, therefore, nucleosomes repeat at intervals of about 200 nucleotide pairs. For example, a diploid human cell with $6.4 \cdot 10^9$ nucleotide pairs contain approximately 30 million nucleosomes. The formation of nucleosomes converts a DNA molecule into a chromatin thread about one-third of its initial length.

Despite the uniform assembly of chromosomal DNA into nucleosomes, a large variety of different chromatin structures are possible in eukaryotic organisms. This variety is based on a large set of reversible covalent modifications of the four histones in the nucleosome core. These modifications include the mono-, di-, and tri-methylation of many different lysine side chains, an important reaction that is incompatible with the acetylation of the same lysines. Specific combinations of the modifications mark each nucleosome with a histone code. The histone code is read when protein modules that are part of a larger protein complex bind to the modified nucleosomes in a region of chromatin. These code-reader proteins then attract additional proteins that catalyze biologically relevant functions.

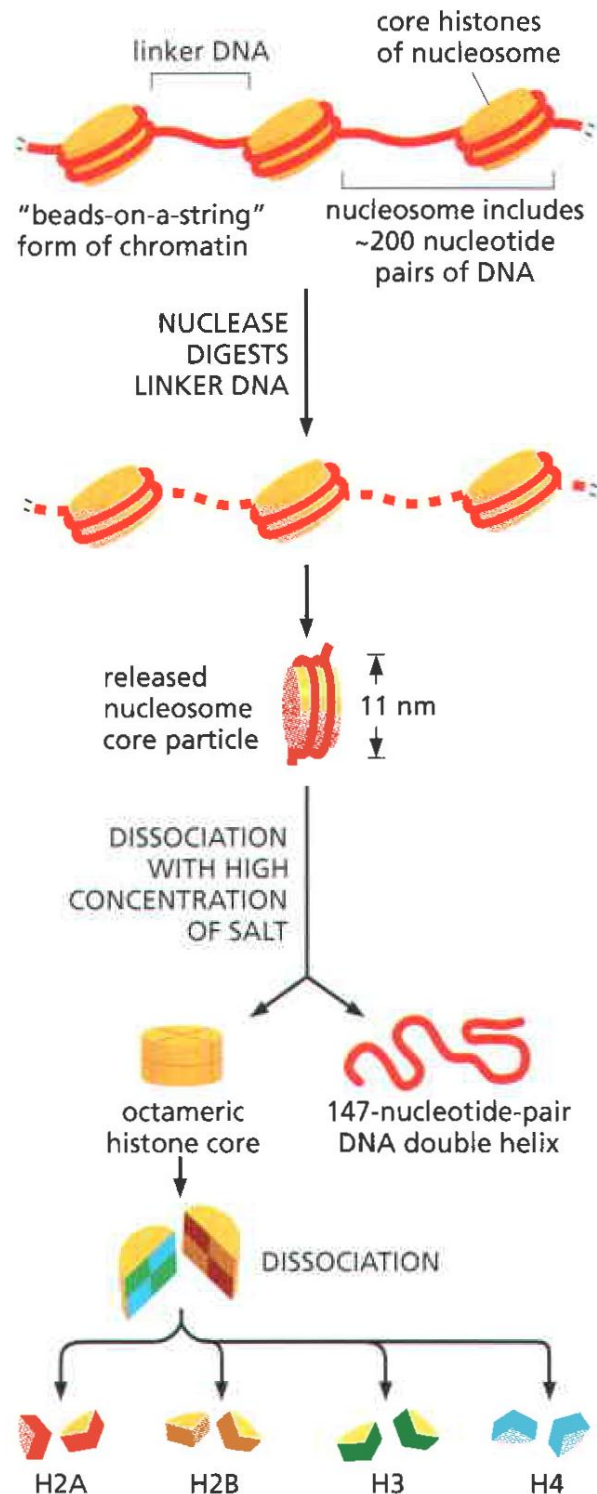


Figure 9.2. Structural organization of the nucleosome (Alberts et al., 2008, p. 212)

A nucleosome contains a protein core made of eight histone molecules. In biochemical experiments, the nucleosome core particle can be released from isolated chromatin by digestion of the linker DNA with a nuclease, an enzyme that breaks down DNA. (The nuclease can degrade the exposed linker DNA but cannot attack the DNA wound tightly around the nucleosome core.) After dissociation of the isolated nucleosome into its protein core and DNA, the length of the DNA that was wound around the core can be determined. This length of 147 nucleotide pairs is sufficient to wrap 1.7 times around the histone core

Although enormously long strings of nucleosomes form on the chromosomal DNA, chromatin in a living cell probably rarely adopts the extended «beads on a string» form. Instead, the nucleosomes are packed on top of one another, generating regular arrays in which the DNA is even more highly condensed – the 30-nm chromatin fiber (**fig. 9.3**). How are nucleosomes packed in? The nucleosome to nucleosome linkages formed by histone tails, most notably the H4 tail (Figure 4-33) constitute one important factor. Another important factor is an additional histone that is often present in a 1-to-1 ratio with nucleosome cores, known as histone H1. This so-called linker histone is larger than the individual core histones. A single histone H1 molecule binds to each nucleosome, contacting both DNA and protein, and changing the path of the DNA as it exits from the nucleosome. H1 pulls nucleosomes together into the 30-nm fiber, a change in the exit path in DNA seems crucial for compacting nucleosomal DNA so that it interlocks to form the 30-nm fiber. Most eukaryotic organisms make several histone H1 proteins of related but quite distinct amino acid sequences.

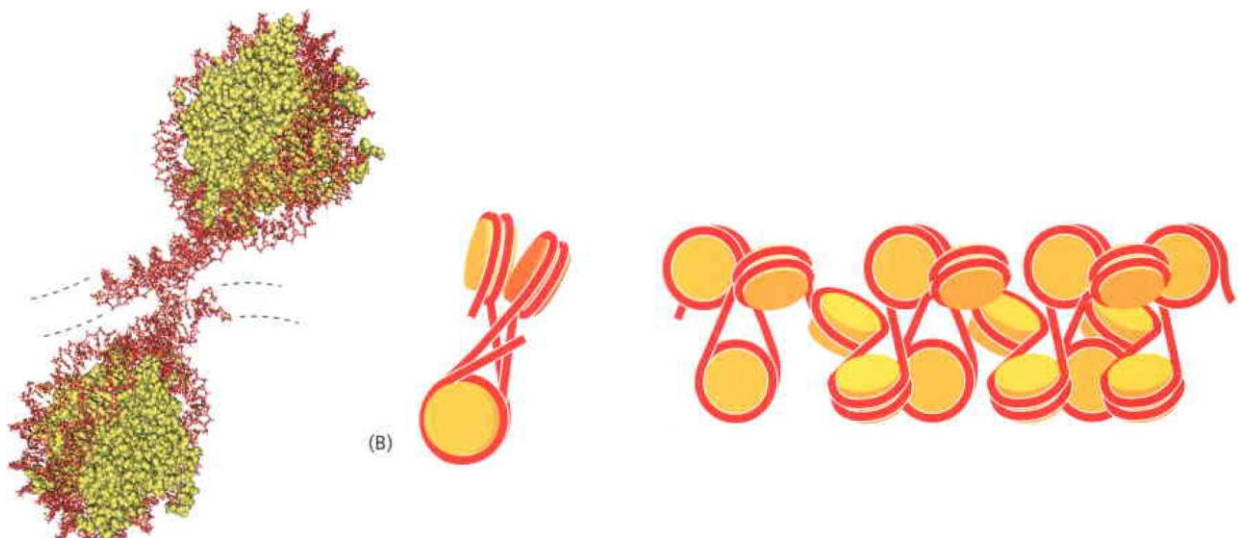


Figure 9.3. A zigzag model for the 30-nm chromatin fiber (Adapted from C.L. Woodcock, *Wat. Struct. Mol. Biol.* 12:639–640, 2005. Alberts et al., 2008, p. 217)

(A) The conformation of two of the four nucleosomes in a tetranucleosome, from a structure determined by x-ray crystallography. (B) Schematic of the entire tetranucleosome; the fourth nucleosome is not visible, being stacked on the bottom nucleosome and behind it in this diagram. (C) Diagrammatic illustration of a possible zigzag structure that could account for the 30-nm chromatin fiber

The third level of packaging is chromomeric (DNA loop domains). This level of DNA compaction is associated with the formation of a transverse loop structure running along an interphase or mitotic chromosome. This structure consists of a huge number (up to 50,000) of looped DNAs, each containing about 60,000 bp. The average size of such loops is 100–150 nm. The base of the loops is attached inside the nucleus to the areas of non-histone proteins, which amount to about 20% of all chromatin proteins. This is a heterogeneous group of proteins that differ from

each other in properties and functions. About 80% of non-histone proteins belong to proteins in the nuclear matrix. Among non-histone proteins, there are regulatory proteins that stimulate or inhibit transcription. Non-histone proteins also include enzymes that participate in the metabolism of nucleic acids (DNA polymerases, DNA topoisomerases, DNA and RNA methylases, RNA polymerases, RNAases and DNAases, etc.), of chromatin proteins (protein kinases, methylases, acetylases, proteases, etc.) and many others.

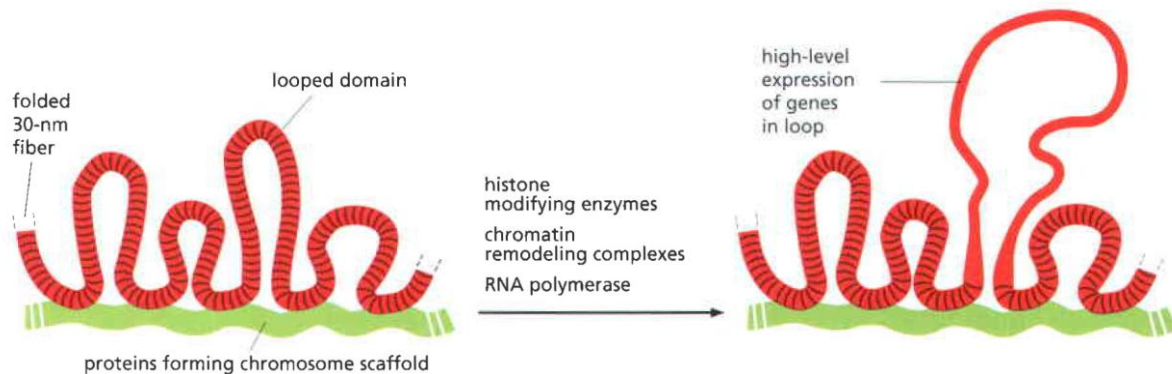
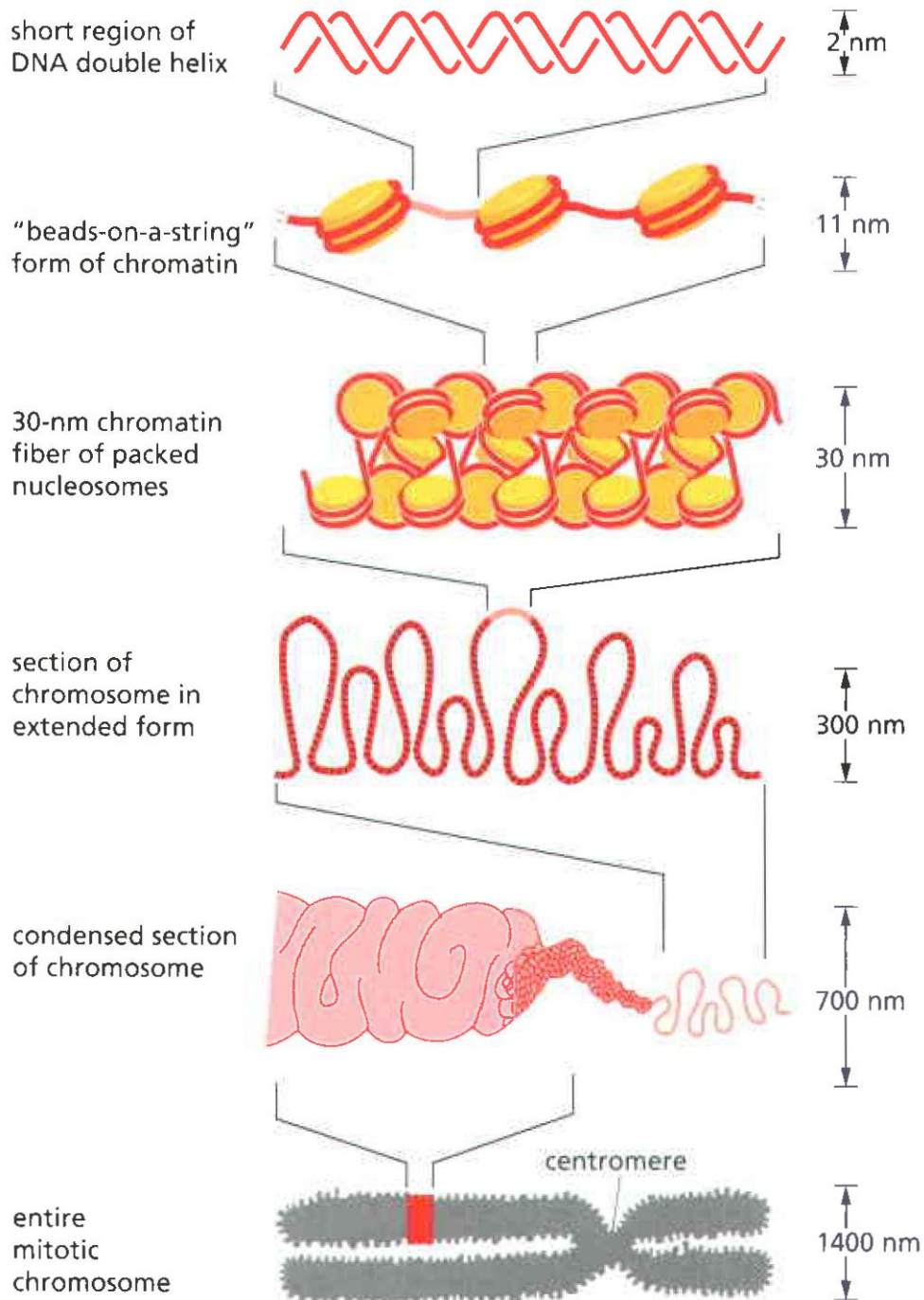


Figure 9.4. A model for the organization of an interphase chromosome (Alberts et al., 2008, p. 236)

A section of an interphase chromosome is shown folded into a series of looped domains, each containing perhaps 50,000–200,000 nucleotide pairs of double-helical DNA condensed into a 30-nm fiber. The chromatin in each individual loop is further condensed that are reversed when the cell requires direct access to the DNA packaged in the loop. In mitotic chromosomes the bases of the chromosomal loops are enriched both in condensins and in DNA topoisomerase II enzymes

Chromonemic level is the fourth level of chromatin packaging. The looped chromosomal DNA is coiled into an even more dense structure due to superspiralization resulting in a filamentous structure, the chromonema that is 0.1–0.2 microns thick. Superspiralization or condensation of chromosomes occurs due to the phosphorylation reaction of histone H1, which binds nucleosome particles with each other. This results in the formation of a typical chromosome at the metaphase stage. It should be noted that the chemical composition of chromatin and chromosomes is the same; these are two different structural states of genetic material (**fig. 9.5**).

Chromosomes are generally decondensed during interphase, so that the details of their structure are difficult to visualize. Notable exceptions are the specialized lampbrush chromosomes of vertebrate oocytes and the polytene chromosomes in the giant secretory cells of insects. Studies of these two types of interphase chromosomes suggest that each long DNA molecule in a chromosome is divided into a large number of discrete domains organized as loops of chromatin, each loop probably consisting of a 30-nm chromatin fiber that is compacted by further folding (**fig. 9.6**). When genes contained in a loop are expressed, the loop unfolds and allows the cell's machinery access to the DNA.



NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH

Figure 9.5. Chromatin packing (Alberts et al., 2008, p. 244)

This model shows levels of chromatin packing postulated to give rise to the highly condensed mitotic chromosome

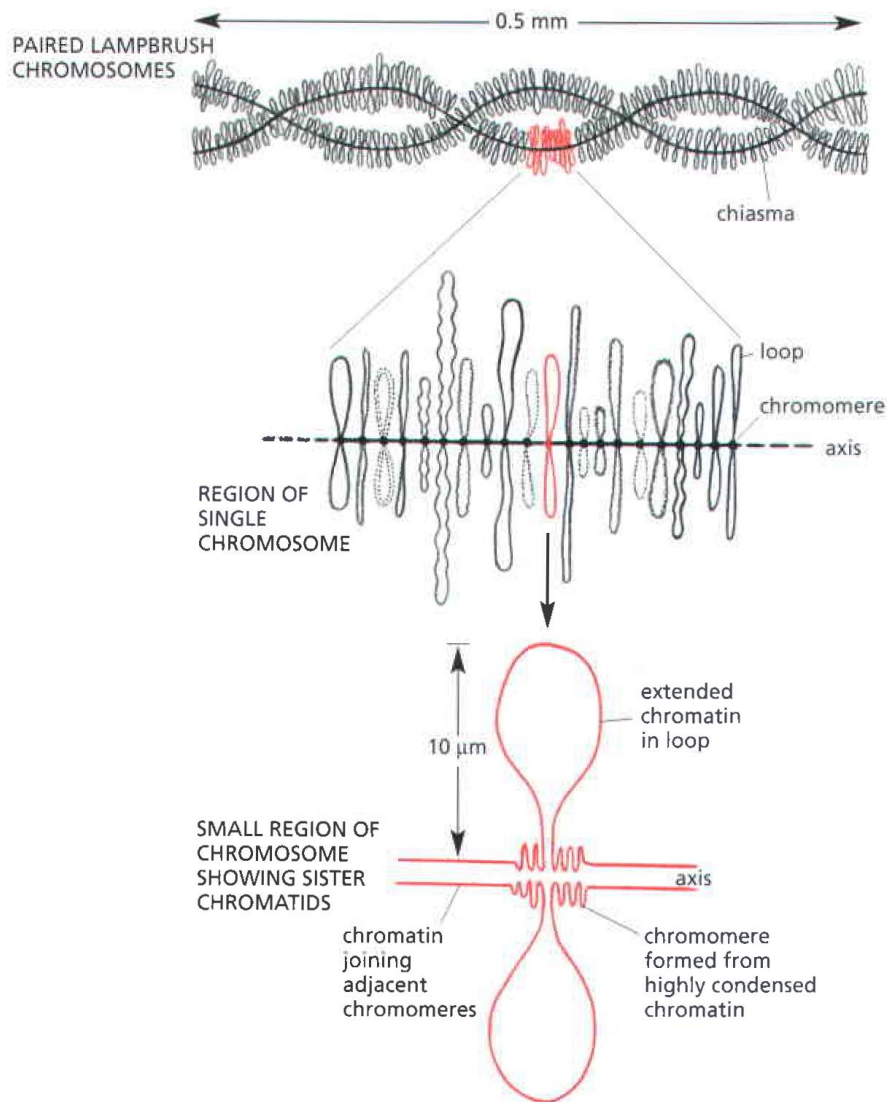


Figure 9.6. A model for the structure of a lamp brush chromosome (Alberts et al., 2008, p. 235) The set of lamp brush chromosomes in many amphibians contains a total of about 10,000 chromatin loops, although most of the DNA in each chromosome remains highly condensed in the chromomeres. Each loop corresponds to a particular DNA sequence. Four copies of each loop are present in each cell, since each of the two major units shown at the top consists of two closely apposed, newly replicated chromosomes

Interphase chromosomes occupy discrete territories in the cell nucleus; that is, they are not extensively intertwined. Euchromatin makes up most of interphase chromosomes and, when not being transcribed, it probably exists as tightly folded 30-nm fibers. However, euchromatin is interrupted by stretches of heterochromatin, in which the 30-nm fibers are subjected to additional packing that usually renders it resistant to gene expression. Heterochromatin exists in several forms, some of which are found in large blocks in and around centromeres and near telomeres. But heterochromatin is also present at many other positions on chromosomes, where it can serve to regulate developmentally important genes.

The interior of the nucleus is highly dynamic, with heterochromatin often positioned near the nuclear envelope and loops of chromatin moving away from

their chromosome territory when genes are very highly expressed. This reflects the existence of nuclear subcompartments, where different sets of biochemical reactions are facilitated by an increased concentration of selected proteins and RNAs. The components involved in forming a subcompartment can self-assemble into discrete organelles such as nucleoli or Cajal bodies; they can also be tethered to fixed structures such as the nuclear envelope.

During mitosis, gene expression shuts down and all chromosomes adopt a highly condensed conformation in a process that begins early in M phase to package the two DNA molecules of each replicated chromosome as two separately folded chromatids. This process is accompanied by histone modifications that facilitate chromatin packing. However, satisfactory completion of this orderly process, which reduces the end-to-end distance of each DNA molecule from its interphase length by an additional factor often, requires condensin proteins.

9.4. Surface apparatus

The nucleus surface apparatus consists of three main components: the nuclear envelope, the peripheral dense plate (lamina) and pore complexes.

The nuclear envelope encloses the DNA and defines the nuclear compartment. This envelope consists of two concentric membranes, which are penetrated by nuclear pore complexes (*fig. 9.7*).

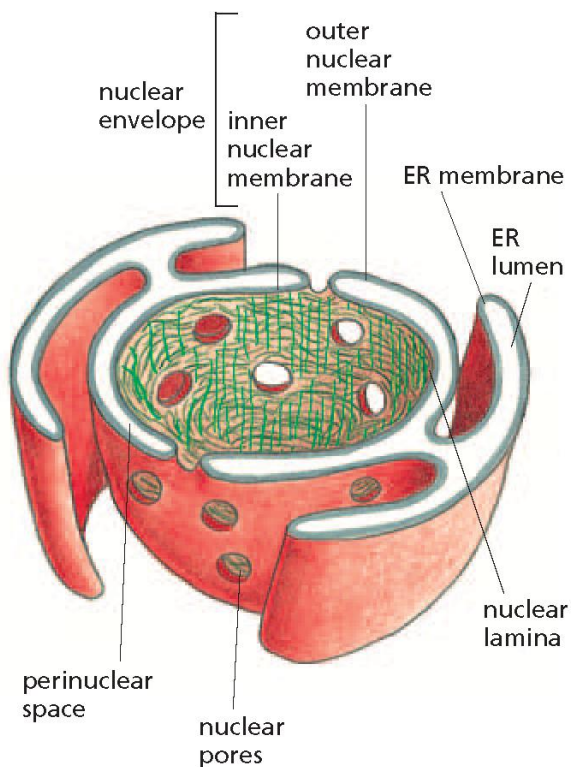


Figure 9.7. The nuclear envelope (Alberts et al., 2008, p. 705)

The double-membrane envelope is penetrated by pores in which NPCs are positioned and is continuous with the endoplasmic reticulum. The ribosomes that are normally bound to the cytosolic surface of the ER membrane and outer nuclear membrane are not shown. The nuclear lamina is a fibrous meshwork underlying the inner membrane

Although the inner and outer nuclear membranes are continuous, they maintain distinct protein compositions. The inner nuclear membrane contains specific proteins that act as anchoring sites for chromatin and for the nuclear lamina, a protein meshwork that provides structural support for the nuclear envelope. The inner membrane is surrounded by the outer nuclear membrane, which is continuous with the membrane of the ER. Like the membrane of the ER (discussed later), the outer nuclear membrane is studded with ribosomes engaged in protein synthesis. The proteins made on these ribosomes are transported into the space between the inner and outer nuclear membranes (the perinuclear space), which is continuous with the ER lumen.

Bidirectional traffic occurs continuously between the cytosol and the nucleus. The many proteins that function in the nucleus, including histones, DNA and RNA polymerases, gene regulatory proteins, and RNA-processing proteins, are selectively imported into the nuclear compartment from the cytosol, where they are made. At the same time, tRNAs and mRNAs are synthesized in the nuclear compartment and then exported to the cytosol. Like the import process, the export process is selective; mRNAs, for example, are exported only after they have been properly modified by RNA-processing reactions in the nucleus. In some cases, the transport process is complex. Ribosomal proteins, for instance, are made in the cytosol and imported into the nucleus, where they assemble with newly made ribosomal RNA into particles. The particles are then exported to the cytosol, where they assemble into ribosomes. Each of these steps requires selective transport across the nuclear envelope.

The inner membrane of the nucleus is connected to the proteins of the dense lamina plate through a system of integral proteins, and the lamina has three types of fibrillar proteins (lamins A, B, C). During mitosis, the lamina proteins are phosphorylated, which leads to the destruction of the nuclear envelope, and the assembly of the lamina occurs in a dephosphorylation reaction, which ensures the integrity of the nuclear envelope.

Nuclear pore complexes. Large, elaborate structures known as nuclear pore complexes (NPCs) perforate the nuclear envelope of all eucaryotes. In animal cells, each NPC has an estimated molecular mass of about 125 million daltons and is composed of about 30 different NPC proteins, or nucleoporins, which are present in multiple copies and are arranged with a striking octagonal symmetry (*fig. 9.8*).

The nuclear envelope of a typical mammalian cell contains 3000–4000 NPCs, and the total traffic that passes through each NPC is enormous: each NPC can transport up to 500 macromolecules per second and can transport in both directions at the same time. How it coordinates the bidirectional flow of macromolecules to avoid congestion and head-on collisions is not known.

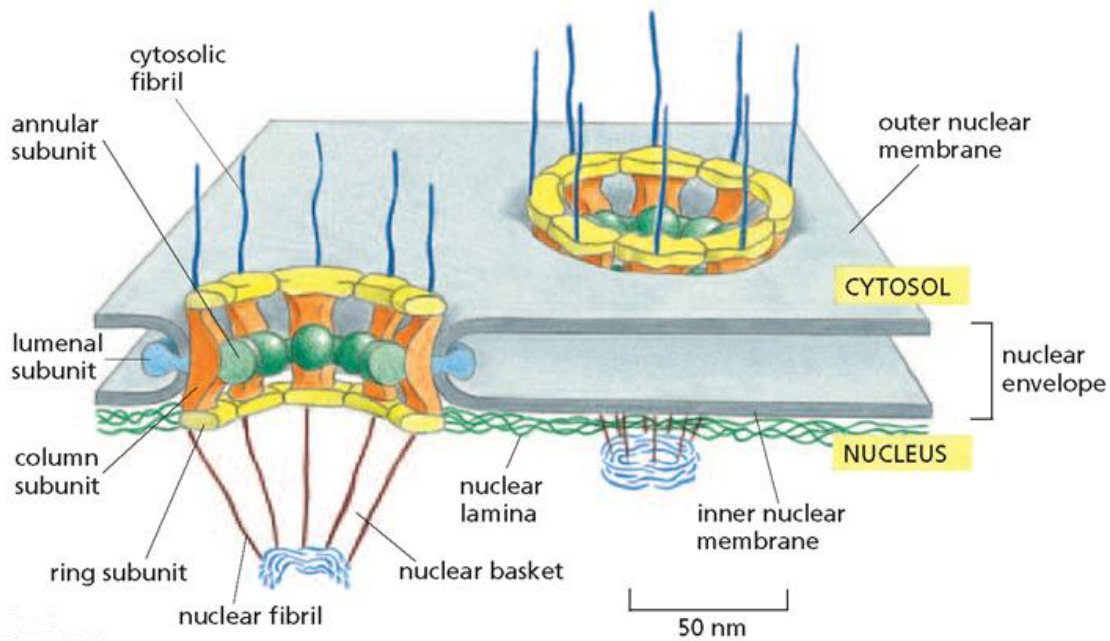


Figure 9.8. The arrangement of NPCs in the nuclear envelope (Alberts et al., 2008, p. 706)

A small region of the nuclear envelope. In cross section, an NPC seems to have four structural building blocks: column subunits, which form the bulk of the pore wall; annular subunits, which are centrally located; luminal subunits, which contain transmembrane proteins that anchor the complex to the nuclear membrane; and ring subunits, which form the cytosolic and nuclear faces of the complex. In addition, fibrils protrude from both the cytosolic and the nuclear sides of the NPC. On the nuclear side, the fibrils converge to form basketlike structures. Immunoelectron microscopic studies show that the proteins that make up the core of the NPC are oriented symmetrically across the nuclear envelope, so that the nuclear and cytosolic sides of the core look identical. In contrast, the proteins that make up the fibrils are different on the cytosolic and nuclear sides of the NPC. The eight-fold rotational and two-fold transverse symmetry of the core NPC explains how such a huge structure can be formed from only about 30 different proteins: many of these proteins are present in 16 copies (or multiples of 16)

Each NPC contains one or more aqueous passages, through which small water-soluble molecules can diffuse passively. Researchers have determined the effective size of these passages by injecting labeled water-soluble molecules of different sizes into the cytosol and then measuring their rate of diffusion into the nucleus. Small molecules (5000 daltons or less) diffuse in so fast that we can consider the nuclear envelope freely permeable to them. Large proteins, however, traverse the NPC much more slowly; the larger a protein, the more slowly it passes through the NPC. Proteins larger than 60,000 dalton's can barely enter by passive diffusion. This size cut-off to free diffusion is thought to result from the NPC structure (**fig. 9.9**). Many NPC proteins that line the central pore contain extensive unstructured regions, which are thought to form a disordered tangle (much like a kelp bed in the ocean), blocking the central opening in the NPC to the passage of large macromolecules, but leaving small openings to allow the diffusion of smaller molecules.

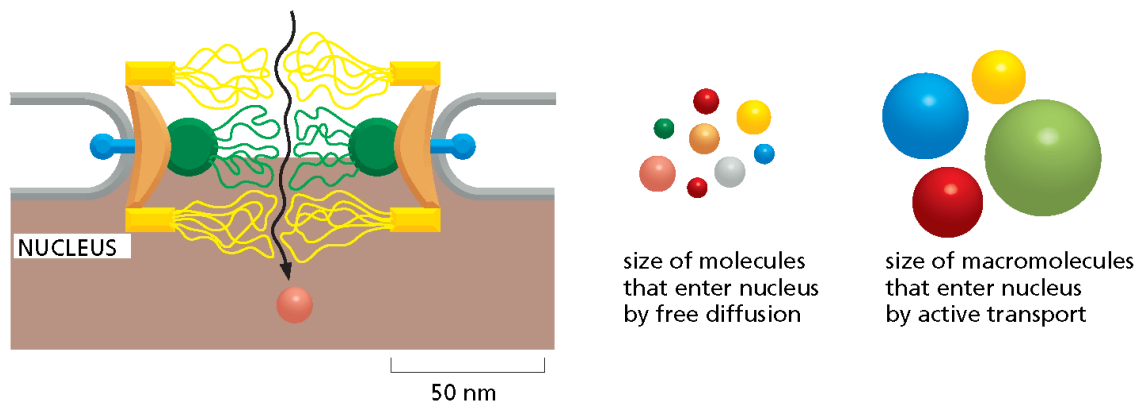


Figure 9.9. A model for the gated diffusion barrier of the NPC (Alberts et al., 2008, p. 706)

The drawing shows a side view of an NPC. Unstructured regions of the proteins lining the central pore form a tangled meshwork, which blocks the passive diffusion of large macromolecules. During active transport, however, even particles up to 39 nm in diameter can pass through NPCs

Because many cell proteins are too large to diffuse passively through the NPCs, the nuclear compartment and the cytosol can maintain different complements of proteins. Mature cytosolic ribosomes, for example, are about 30 nm in diameter and thus cannot diffuse through the NPC, confining protein synthesis to the cytosol. But how does the nucleus export newly made ribosomal subunits or import large molecules, such as DNA and RNA polymerases, which have subunit molecular weights of 100,000–200,000 Daltons? Many other protein and RNA molecules bind to specific receptor proteins that ferry large molecules actively through NPCs.

9.5. Nucleolus

Separate bodies within the nucleus were first recognized by the Italian naturalist Felice Fontana in 1774 and named «nucleoli».

The nucleolus is the largest structure in the nucleus of eukaryotic cells. It is best known as the site of ribosome biogenesis. Nucleoli also participate in the formation of signal recognition particles and play a role in the cell's response to stress. Nucleoli are made of proteins, DNA and RNA and form around specific chromosomal regions called nucleolar organizing regions. The nucleolus ultrastructure can be seen through an electron microscope, while the organization and dynamics can be studied through fluorescent protein tagging.

The main component of the nucleolus is protein: it accounts for up to 70–80% of dry weight. The nucleolus contains all components necessary for the formation of ribosomal subunits (rRNA genes, RNA itself, enzymes for RNA synthesis and processing, ribosomal proteins imported from cytoplasm). Nucleoli are associated topographically with certain zones on special nucleolus-forming chromosomes. Ribosomal RNA genes are localized in nucleolar organizers. Active ribosomal genes are shown in **fig. 9.10**.

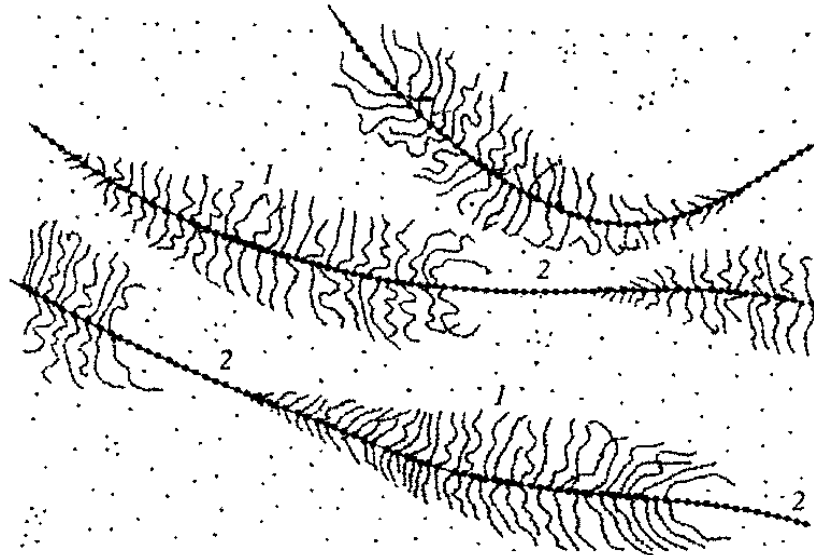


Figure 9.10. rRNA genes (Chentsov, 2015, p. 162)

1 – transcribable sequence; 2 – spacer

The ribosomal gene consists of a non-transcribed DNA sequence (the spacer) and a DNA section where rRNA molecules are located in the process of their elongation (the transcription unit) (*fig. 9.11*).

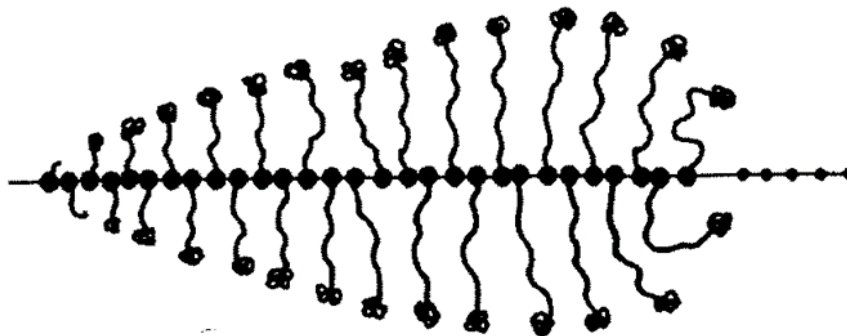


Figure 9.11. Structure of one transcription unit (rRNA gene) (Chentsov, 2015, p. 164)

The large granules on the DNA filament are RNA polymerase I molecules responsible for the synthesis of rRNA, and the lateral curved filaments are transcripts consisting of the synthesized RNA molecules. The longest transcripts are at one end of the «herringbone» and correspond to the 45S precursor of rRNA. The synthesis of rRNA starts at the end of a section with short side filaments and ends at the section with long RNA filaments. Between the transcription units, there are regions of spacers that are not transcribed.

Three major components of the nucleolus are recognized: the fibrillar center (FC), the dense fibrillar component (DFC), and the granular component (GC) (*fig. 9.12*). Transcription of the rDNA occurs in the FC. The DFC contains the protein fibrillarin, which is important in rRNA processing. The GC contains the protein nucleophosmin, which is also involved in ribosome biogenesis.

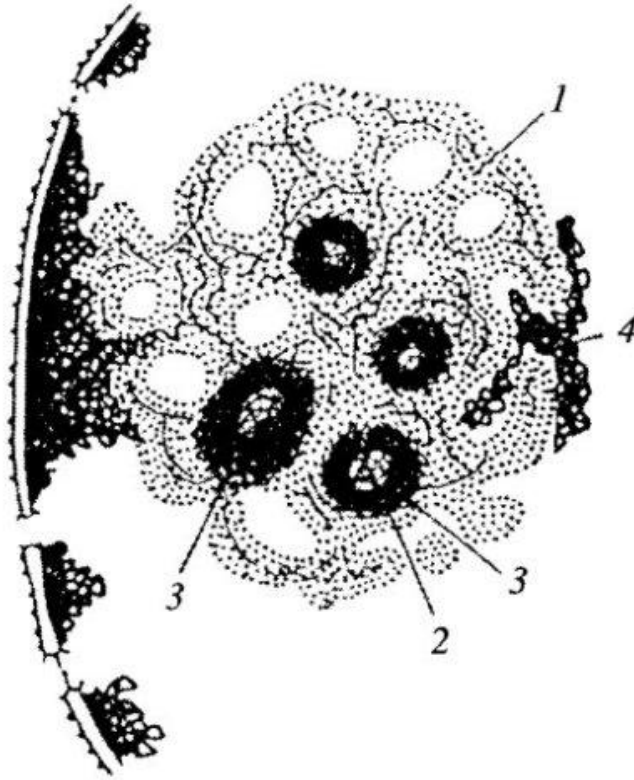


Figure 9.12. Schematic of nucleolus components (Chentsov, 2015, p. 168)

1 – granular component (nucleolonem); 2 – fibrillar centers; 3 – dense fibrillar component; 4 – perinucleolar chromatin

There are several structural types of nucleoli: reticular or nucleolonemic, compact, annular, residual, segregated. Reticular or nucleolonemic nucleolus type is characteristic of most cells, such nucleoli have a nucleolonemic structure with abundance of granules and fibrillar dense material. Nucleoli of this type are found in most eukaryotic cells and in giant polytene chromosomes of dipteran insects.

The compact type of nucleolus differs from the previous one by having a less pronounced nucleolonema and a higher frequency of occurrence of fibrillar centers. Such nucleoli are typical for actively reproducing cells (plant meristems, cells of tissue culture).

Ring-shaped nucleoli are found in animal cells. They take the form of a ring with an optically light fibrillar center surrounded by RNP fibrils and granules. The size of these nucleoli is about 1 μm . They are typical for lymphocytes, endotheliocytes, i.e. for cells with a relatively low transcription level.

Residual nucleoli are typical for cells that have completely lost the ability to synthesize rRNA (normoblasts, differentiated enterocytes, cutaneous epithelium cells, etc.). They have a small size and are surrounded by condensed chromatin. In some cases, they may be reactivated and converted to a compact or reticular form.

Segregated nucleoli are typical for cells treated with various antibiotics or chemicals that stop rRNA synthesis. They have a relatively small volume and comprise separated large fibrillar centers and a granular-fibrillar component (*fig. 9.13*).

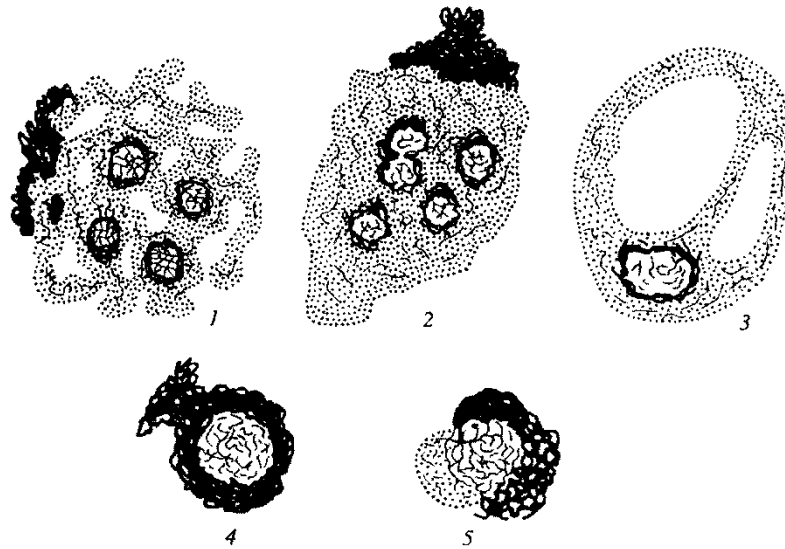


Figure 9.13. Structural types of nucleoli (Chentsov, 2015, p. 174)
1 – reticular; 2 – compact; 3 – ring-shaped; 4 – residual; 5 – segregated

In germinal cells, additional replication of rRNA genes may occur, which produces extra-chromosome copies of rRNA genes. This results in the formation of additional nucleoli that are not structurally connected with nucleolus-forming chromosomes. This phenomenon is referred to as amplification of nucleoli.

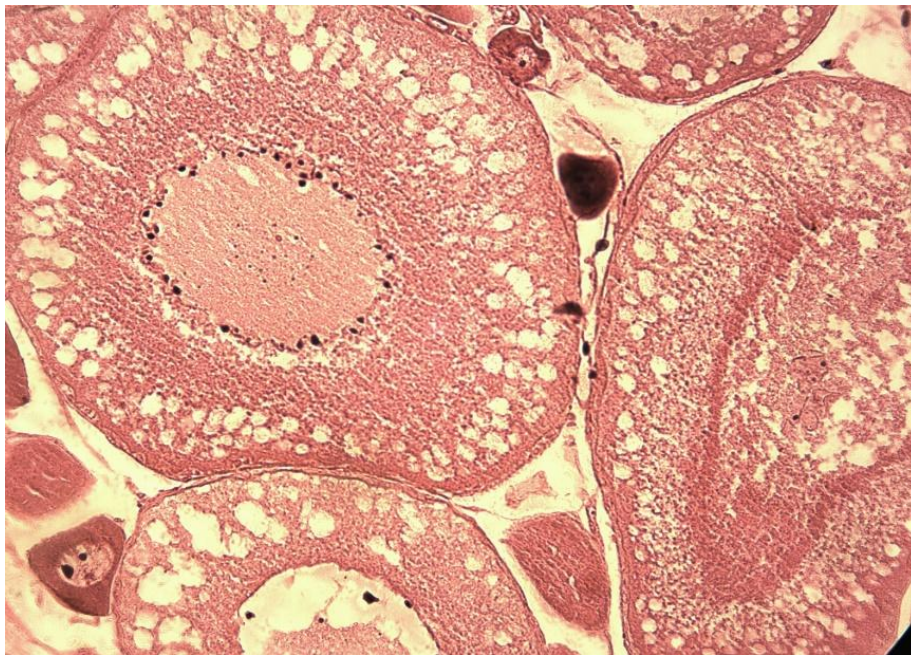


Figure 9.14. Nucleoli in bony fish eggs. The phenomenon of amplification
The preparation is courtesy of Yu.S. Chentsov (Moscow State University, Cytology Department). The digital micrograph of the preparation was made by the author with a Vision CAM camera for the Meiji Techno trinocular microscope using an integrated adapter and a C-mount connector (magnification x 600)

The biological significance of amplification is that in the early stages of embryogenesis, a huge number of ribosome precursors is required, and they are synthesized in the cell on additional matrices of these amplified nucleoli. Amplification is a temporary phenomenon, which does not affect the constancy of the genome, because after the period of oocyte maturation in two successive divisions these additional nucleoli degrade.

CONTROL TESTS

Read attentively each assignment (question) and choose one correct answer from the choices offered below.

1. The chromosomal region where rRNA synthesis occurs and ribosomes' sub-particles are formed is called:

- a) the nucleolus;
- b) the nucleus;
- c) the nuclear matrix.

2. The liquid phase of the cell nucleus in which many processes related to both nuclear metabolism and intra-nuclear transport of proteins and RNA is called:

- a) karyoplasm;
- b) karyotype;
- c) karyolemma.

3) Partially or completely decompensated chromatin is characterized by:

- a) synthetic activity;
- b) mitotic activity;
- c) metabolic rest state.

4. Permanently condensed chromatin areas in interphase nuclei are commonly called:

- a) constitutive heterochromatin;
- b) facultative heterochromatin;
- c) euchromatin.

5. Complex molecules of deoxyribonucleoprotein (DNP), consisting of DNA associated with histones, are called:

- a) nucleohistones;
- b) nucleomeres;
- c) nucleotides.

TOPICS FOR INDEPENDENT WORK

Independent work includes the study of the following topics:

Topic 1: The role of the nucleus in cell life and its importance in transferring information from DNA to the protein. Main functions of the nucleus: transcription, reduplication and redistribution of genetic material. Interphase nucleus.

Topic 2: Chromatin, its chemical characteristics. Chromatin varieties: despiralized euchromatin, condensed heterochromatin and facultative heterochromatin. Functional significance of chromatin types.

Topic 3: Chromatin proteins: histones and non-histone proteins. The function of histones as regulators of transcription and stacking of DNA molecules.

Topic 4: Nucleosomal, nucleomeric and chromonemic DNA packaging levels.

Topic 5: The structure and functions of the nucleus surface apparatus. Features of nuclear membranes. Lamina, the skeletal plate. The structure of pore complexes.

THE CELL CYCLE

The cell cycle is the essential mechanism by which all living things reproduce. The details of the cell cycle vary from organism to organism and at different times in an organism's life. Certain characteristics, however, are universal. The minimum set of processes that a cell has to perform are those that allow it to accomplish its most fundamental task: the passing on of its genetic information to the next generation of cells. To produce two genetically identical daughter cells, the DNA in each chromosome must first be faithfully replicated to produce two complete copies, and the replicated chromosomes must then be accurately distributed (segregated) to the two daughter cells, so that each receives a copy of the entire genome (**fig. 10.1**).

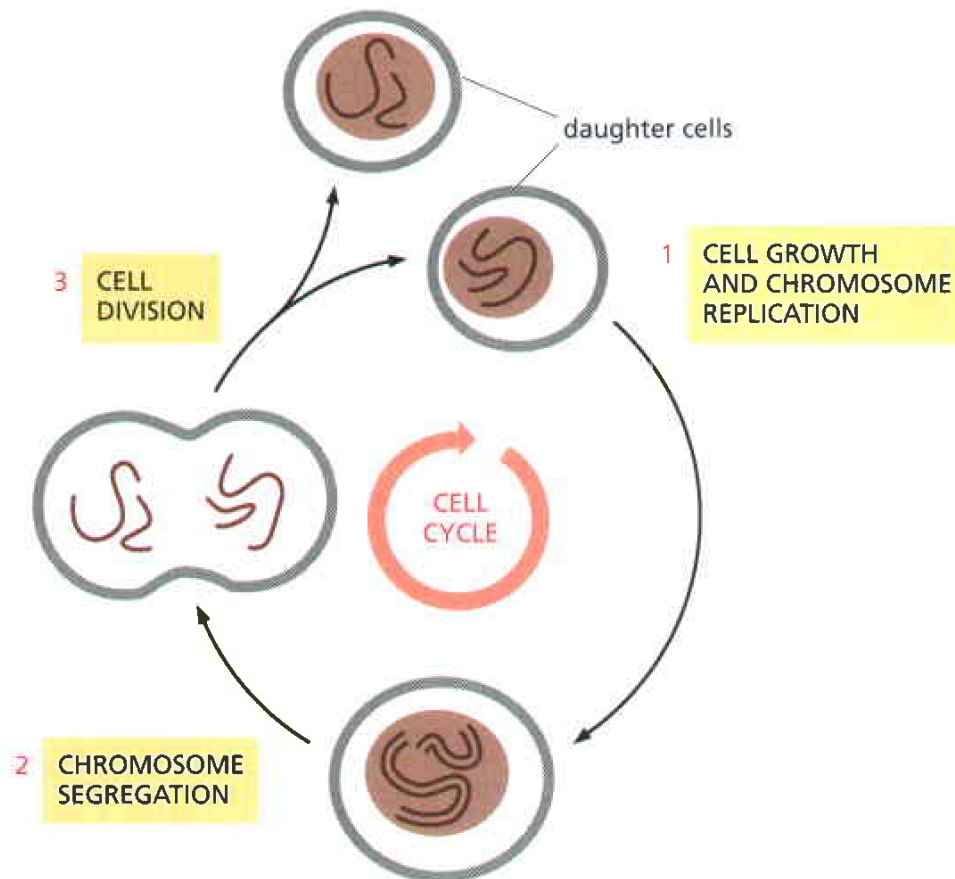


Figure 10.1. The cell cycle (Alberts et al., 2008, p. 1054)

The division of a hypothetical eukaryotic cell with two chromosomes is shown to illustrate how two genetically identical daughter cells are produced in each cycle. Each of the daughter cells will often continue to divide by going through additional cell cycles

Eukaryotic cells have evolved a complex network of regulatory proteins, known as the cell-cycle control system, that governs progression through the cell cycle. The core of this system is an ordered series of biochemical switches that initiate the main events of the cycle, including chromosome duplication and segregation.

The most basic function of the cell cycle is to duplicate accurately the vast amount of DNA in the chromosomes and then segregate the copies precisely into two genetically identical daughter cells. These processes define the two major phases of the cell cycle. Chromosome duplication occurs during S phase (S for DNA synthesis), which requires 10–12 hours and occupies about half of the cell-cycle time in a typical mammalian cell. After S phase, chromosome segregation and cell division occur in M phase (M for mitosis), which requires much less time (less than an hour in a mammalian cell). M phase comprises two major events: nuclear division, or mitosis, during which the copied chromosomes are distributed into a pair of daughter nuclei; and cytoplasmic division, or cytokinesis, when the cell itself divides in two. Thus, the eukaryotic cell cycle is traditionally divided into four sequential phases: G₁, S, G₂, together are called interphase and mitosis and cytokinesis (**fig. 10.2**).

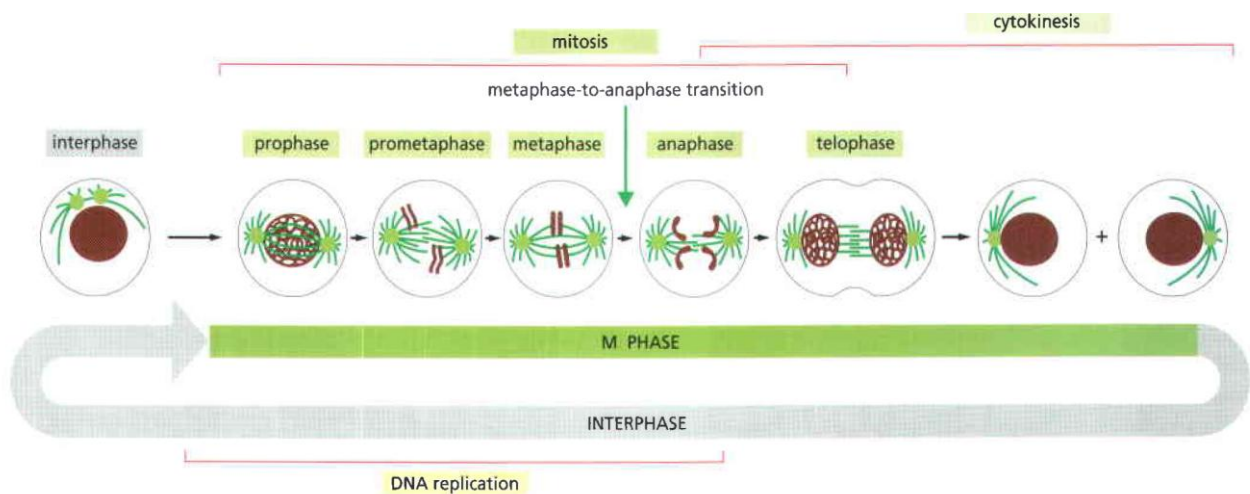


Figure 10.2. The events of eukaryotic cell division as seen under a microscope (Alberts et al., 2008, p. 1055)

The easily visible processes of nuclear division (mitosis) and cell division (cytokinesis), collectively called M phase, typically occupy only a small fraction of the cell cycle. The other, much longer, part of the cycle is known as interphase, which includes S phase and the gap phases. The five stages of mitosis are shown: an abrupt change in the biochemical state of the cell occurs at the transition from metaphase to anaphase. A cell can pause in metaphase before this transition point, but once it passes this point, the cell carries on to the end of mitosis and through cytokinesis into interphase.

10.1. Interphase

The pre-synthetic period, or the G1 phase, is the period before DNA synthesis starts. It is characterized by predominant cytoplasm growth. This period is the longest one in the cycle. It takes from 10–18 hours to several days. During the G1 period, intensive cell metabolism takes place; mitochondria, chloroplasts (in plants), ER, lysosomes, Golgi complex, vacuoles and secretory vesicles are formed. The nucleus produces all types of RNA, subunits of ribosomes and complete ribosomes are formed, structural and functional proteins are synthesized.

What is the trigger for cells to enter the pre-synthetic cycle? It is a multitude of growth factors that induce cells to reproduce. These factors can be either own products of these cells (autocrine stimulation) or of other neighboring cells (paracrine stimulation), or even of other organs' cells (hormonal stimulation). Growth factors interact on cell membranes with their receptors and transmit a signal through secondary messengers (e.g., cAMP) to the intracellular cascade system.

The most important period is the synthetic period (S-period), when DNA synthesis occurs. Eukaryotic cells have a polyreplicon organization of chromosomes. This means that along the length of chromosomal DNA, there are many independent replication sites, replicons. Each chromosome is characterized by high stability of the replication order along its length. DNA synthesis along the length of an individual chromosome occurs asynchronously, unevenly. Active replicons in an individual chromosome are collected into group clusters, replication units. Replicating units, together with nuclear matrix proteins and replication enzymes, form special zones called clusterosomes, i.e. zones where DNA synthesis takes place. During the S period, duplication of centrioles occurs in animal cells. As this takes place, the mother centriole remains the center of cytoplasmic microtubules formation. As a result of the duplication process, four centriolar cylinders in the form of a diplosome appear in the cell at the end of the S period.

The post-synthetic period, or the G2 period. During this period, a slow, harmonious increase in nucleus and cytoplasm volume is observed. Intensive biosynthesis processes take place along with the division of mitochondria and chloroplasts, and the mitotic spindle begins to form. In animal cells, at this point satellites disappear on the mother diplosome, centrioles are covered with a fibrillar halo, from which mitotic microtubules begin to assemble in the mitosis prophase. At the same time, the cytoplasmic microtubules are dismantled. The post-synthetic period is followed by nuclear division known as karyokinesis.

10.2. Mitosis

Following the completion of S phase and transition through G2, the cell undergoes the dramatic upheaval of M phase.

An open form of mitosis, orthomitosis, whereby the nuclear envelope breaks down completely, occurs in the cells of animals, some protozoans and higher plants. This form of mitosis, in turn, is characterized by astral and anastral types of the achromatic mitotic spindle. The astral (convergent) type of spindle is typical for animal cells. Polar bodies, or centrosomes, are the microtubule organizing centers; they constitute a small area to which the microtubules converge. Centrosomes that contain centrioles are usually located at the poles of astral spindles. Radial microtubules that form star-like zones (cytasters) fan out from the spindle poles. (fig. 10.3).

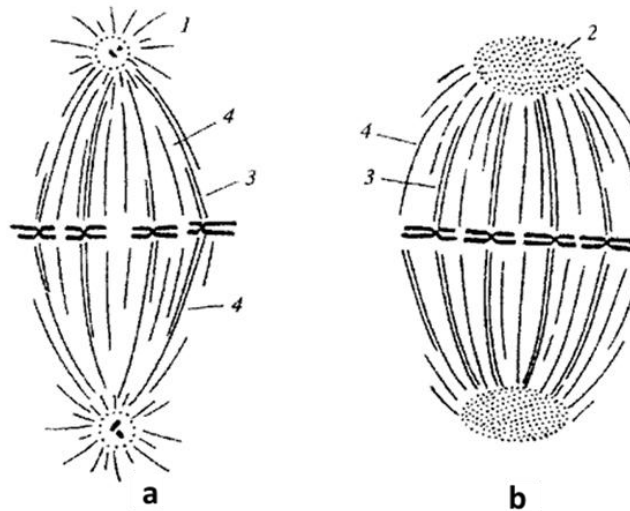


Figure 10.3. Astral (a) and anastral (b) type of mitotic spindle (Chentsov, 2015, p. 428)
1 – cytaster; 2 – «polar caps»; 3 – kinetochoric microtubules; 4 – polar microtubules

The mitotic figure of anastral (divergent) type has no cytasters at the poles. Here, the polar spindle areas are wide, have the shape of unstructured amorphous zones («polar caps») and do not include centrioles. In this case, the spindle microtubules diverge in a wide front from the entire polar cap zone. This type of spindle is typical for dividing cells of higher plants.

Chromosome kinetochores (**fig. 10.4**) are important structures participating in mitotic cell division. Kinetochores are compound complexes consisting of many proteins. Morphologically they are very similar and have the same structure in all eukaryotic cells. Kinetochores have three layers: the inner dense layer adjacent to the chromosome body, the middle loose layer, and the outer dense layer. There is a large number of fibrils protruding from the outer layer that form a fibrous crown of the kinetochore. The functional role of kinetochores is to bind sister chromatids, to fix mitotic microtubules, to regulate the separation of chromosomes and to move chromosomes during mitosis with the participation of microtubules.

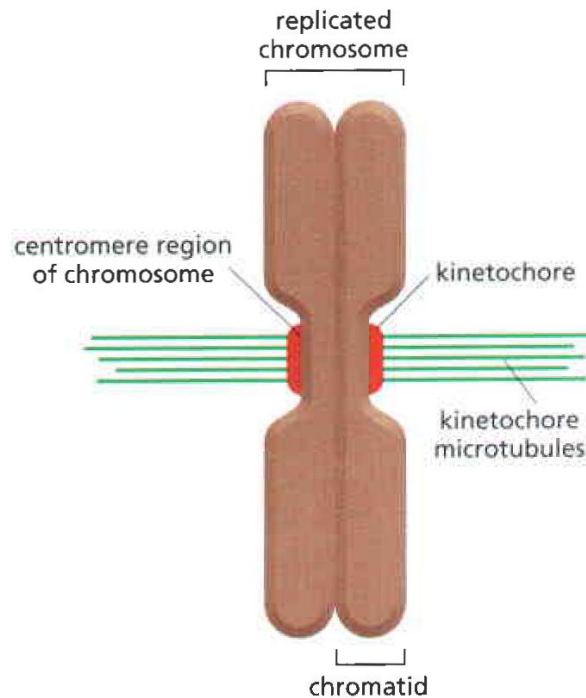


Figure 10.4. The kinetochore (Alberts et al., 2008, p. 1083)

A drawing of a metaphase chromosome showing its two sister chromatids attached to the plus ends of kinetochore microtubules. Each kinetochore forms a plaque on the surface of the centromere. The number of microtubules bound to a metaphase kinetochore varies from 1 in budding yeast to 40 in some mammalian cells

Mitosis is traditionally divided into five stages: prophase, prometaphase, metaphase, anaphase, and telophase – defined primarily on the basis of chromosome behavior as seen in a microscope. As mitosis is completed, the second major event of M phase – cytokinesis – divides the cell into two halves, each with an identical nucleus.

Mitosis is a continuous process, and phase change occurs very gradually. The duration of individual mitosis phases varies, the shortest of them is the anaphase.

The prometaphase involves a constant movement of chromosomes, or metakinesis, in which they approach the poles or move away from them to the centre of the spindle until they take the middle position typical of the metaphase (chromosome congression). The chromosomes are moved by means of microtubules. Separate microtubules extending from the poles accidentally reach one of the chromosome kinetochores and bind to it, they are actually «captured» by the kinetochore. The chromosome then slides rather quickly, at a speed of about 25 $\mu\text{m}/\text{min}$, along the microtubule towards its minus end. This causes the chromosome to approach the pole from which this microtubule was formed. It is believed that a motor protein similar to the cytoplasmic dynein found in the crown of kinetochores is responsible for chromosome movement.

As a result of this primary prometaphase movement, chromosomes are brought in a random fashion closer to the spindle poles, where the assembly of new

microtubules continues. The growing plus ends of the microtubule bundle are «captured» by the kinetochore crown area. As microtubules continue to grow, the chromosome will move to the center of the spindle, away from the pole. At this point, microtubules also grow from the opposite pole to the kinetochore of the sister chromatid, and their bundle begins to pull the chromosome to the opposite pole. As a result, the chromosomes that make small movements towards one or the other pole gradually take the middle position in the spindle and line up into a metaphase plate, thus, the true metaphase begins.

Anaphase begins with the separation of all chromosomes at once in the centromere regions. At this moment, simultaneous degradation takes place of the centromeric cohesin proteins which until then served to bind sister chromatids. The speed of chromosome motion during anaphase is uniform, it can reach 0.5–2 $\mu\text{m}/\text{min}$. When chromosomes move, they change their orientation and often take on a V shape. The pointed parts of V-shaped chromosomes are directed towards spindle poles, and the arms are turned towards the center of the spindle.

The actual splitting of chromosomes involves two processes (*fig. 10.5*).

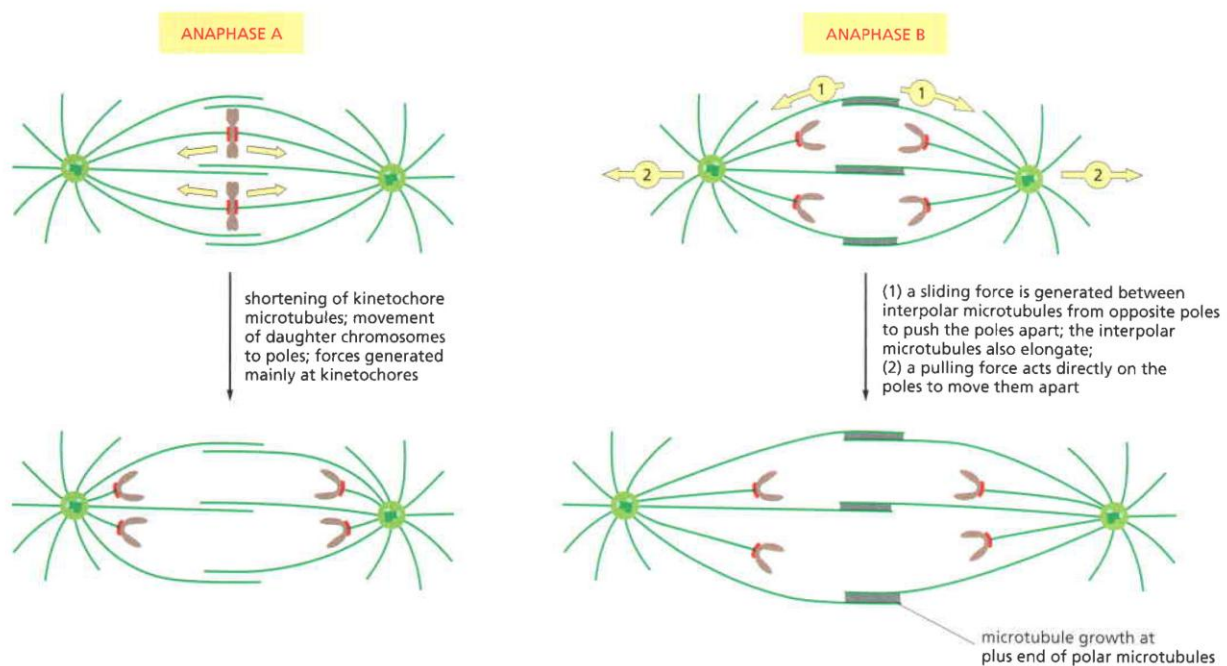


Figure 10.5. The major forces that separate sister chromatids at anaphase in mammalian cells (Alberts et al., 2008, p. 1089)

Chromosome movement toward the poles in anaphase A depends on the depolymerization of kinetochore microtubules and poleward microtubule flux. In anaphase B, the two spindle poles move apart. Two separate forces are thought to be responsible for anaphase B: the elongation and sliding of the interpolar microtubules past one another in the central spindle push the two poles apart, and motor proteins attached to the plasma membrane near each spindle pole act on astral microtubules to pull the poles away from each other, toward the cell surface

The first one, anaphase A, is the splitting of the chromosome due to the shortening of the kinetochoric bundles of microtubules. Disassembly of

microtubules takes place mainly from the plus end of the microtubules, where they are connected to the kinetochore, and the chromosome moves towards the minus end of the microtubules, which is located in the centrosome region. The movement of chromosomes depends on the presence of ATP and sufficient concentration of Ca^{+2} ions. Kinetochore dynein pulls the chromosome to the pole. The second process, anaphase B, is the divergence of the chromosomes together with the poles due to the extension of interpolar microtubules. This part of anaphase is driven by two distinct mechanisms: opposite polarity microtubules are pushed by bifunctional kinesin motor molecules in the center of the spindle, and the astral microtubules are displaced using cytoplasmic dynein localized at the periphery of the cell under the plasmalemma.

Telophase begins when the chromosomes stop moving and it ends with the start of the reconstruction of new interphase nuclei. In the early telophase, the chromosomes begin to decondense and increase in volume. At the points of their contact with cytoplasmic membrane vesicles, a new nuclear envelope begins to form, first on lateral surfaces and then in the centromeric and telomeric areas of chromosomes. When the nuclear envelope closes, new nuclei begin to form.

Karyokinesis is followed by cytokinesis, the division of the cell body. In animal cells, cytokinesis is accomplished by means of constriction when two new structures are formed: the residual body and the contractile ring. After cytokinesis, new cells enter the G1 stage of the cell cycle. The biological significance of mitosis lies in fact that the daughter cells formed in this process have hereditary information, which is qualitatively and quantitatively identical to the mother cell's information. Mitosis ensures that karyotype remains constant in a number of cell generations. It also serves as a mechanism for growth and development processes in an organism, as well as for regeneration and asexual reproduction processes.

From a regulatory point of view, mitosis can be divided into two major parts, each governed by distinct components of the cell-cycle control system. First, an abrupt increase in M-Cdk activity at the G2/M checkpoint triggers the events of early mitosis (prophase, prometaphase, and metaphase). During this period, M-Cdk and several other mitotic protein kinases phosphorylate a variety of proteins, leading to the assembly of the mitotic spindle and its attachment to the sister chromatid pairs. The second major part of mitosis begins at the metaphase-to-anaphase transition, when the APC/C triggers the destruction of securin, liberating a protease that cleaves cohesin and thereby initiates separation of the sister chromatids. The APC/C also triggers the destruction of cyclins, which leads to Cdk inactivation and the dephosphorylation of Cdk targets, which is required for all events of late M phase, including the completion of anaphase, the disassembly of the mitotic spindle, and the division of the cell by cytokinesis.

10.3. Cytokinesis

The final step in the cell cycle is cytokinesis, the division of the cytoplasm. In a typical cell, cytokinesis accompanies every mitosis, although some cells, such as some mammalian hepatocytes and heart muscle cells, undergo mitosis without cytokinesis and thereby acquire multiple nuclei. In most animal cells, cytokinesis begins in anaphase and ends shortly after the completion of mitosis in telophase.

The first visible change of cytokinesis in an animal cell is the sudden appearance of a pucker, or cleavage furrow, on the cell surface. The furrow rapidly deepens and spreads around the cell until it completely divides the cell in two. In animal cells and many unicellular eucaryotes, the structure underlying this process is the contractile ring – a dynamic assembly composed of actin filaments, myosin II filaments, and many structural and regulatory proteins. During anaphase, the ring assembles just beneath the plasma membrane (*fig. 10.6*).

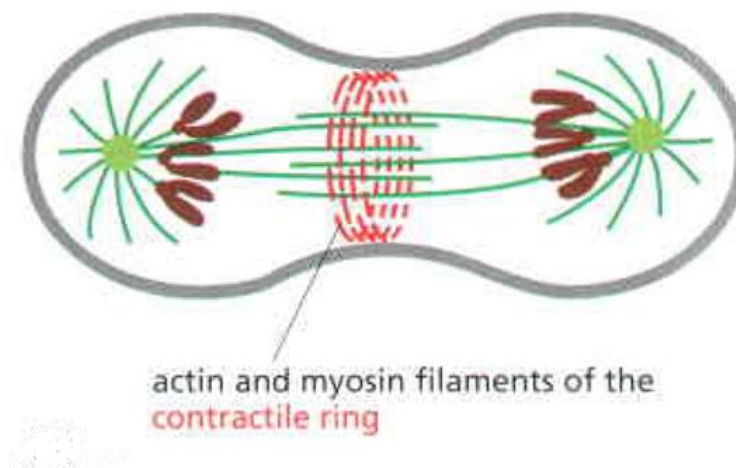


Figure 10.6. Cytokinesis in animal cells (Alberts et al., 2008, p. 1093)

(A) The actin-myosin bundles of the contractile ring are oriented as shown, so that their contraction pulls the membrane inward

The ring gradually contracts, and, at the same time, fusion of intracellular vesicles with the plasma membrane inserts new membrane adjacent to the ring. This addition of membrane compensates for the increase in surface area that accompanies cytoplasmic division. When ring contraction is completed, membrane insertion and fusion seal the gap between the daughter cells. Thus, cytokinesis can be considered to occur in four stages: initiation, contraction, membrane insertion, and completion.

Higher-plant cells are enclosed by a semirigid cell wall. Rather than a contractile ring dividing the cytoplasm from the outside in, the cytoplasm of the plant cell is partitioned from the inside out by the construction of a new cell wall, called the cell plate, between the two daughter nuclei (*fig. 10.7*). The assembly of the cell plate begins in late anaphase and is guided by a structure called the

phragmoplast, which contains microtubules derived from the mitotic spindle. Motor proteins transport small vesicles along these microtubules from the Golgi apparatus to the cell center. These vesicles, filled with polysaccharide and glycoproteins required for the synthesis of the new cell wall, fuse to form a disclike, membrane-enclosed structure called the early cell plate. The plate expands outward by further vesicle fusion until it reaches the plasma membrane and the original cell wall and divides the cell in two. Later, cellulose microfibrils are laid down within the matrix of the cell plate to complete the construction of the new cell wall.

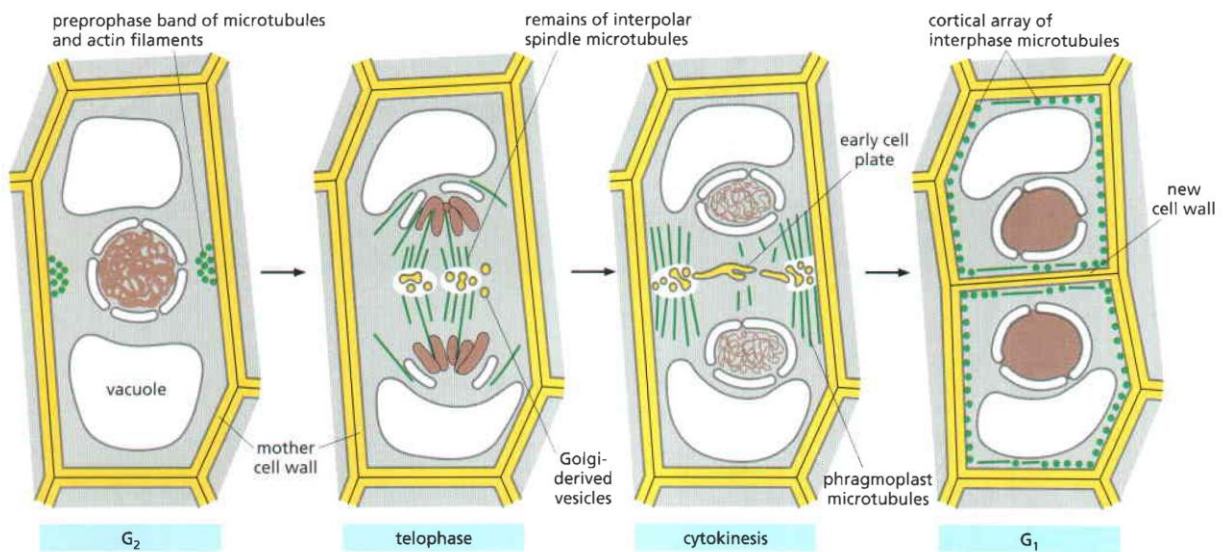


Figure 10.7. Cytokinesis in a higher plant cell (Alberts et al., 2008, p. 1098)

The division plane is established before M phase by a band of microtubules and actin filaments (the preprophase band) at the cell cortex. At the beginning of telophase, after the chromosomes have segregated, a new cell wall starts to assemble inside the cell at the equator of the old spindle. The interpolar microtubules of the mitotic spindle remaining at telophase form the phragmoplast. The plus ends of these microtubules no longer overlap but end at the cell equator. Golgi-derived vesicles, filled with cell-wall material, are transported along these microtubules and fuse to form the new cell wall, which grows outward to reach the plasma membrane and original cell wall. The plasma membrane and the membrane surrounding the new cell wall fuse, completely separating the two daughter cells.

10.4. Cell cycle regulation

The cell-cycle control system is based on a connected series of biochemical switches, each of which initiates a specific cell-cycle event. This system of switches possesses many important engineering features that increase the accuracy and reliability of cell-cycle progression. First, the switches are generally binary (on/ off) and launch events in a complete, irreversible fashion. It would clearly be disastrous, for example, if events like chromosome condensation or nuclear envelope breakdown were only partially initiated or started but not completed.

Second, the cell-cycle control system is remarkably robust and reliable, partly because backup mechanisms and other features allow the system to operate effectively under a variety of conditions and even if some components fail. Finally, the control system is highly adaptable and can be modified to suit specific cell types or to respond to specific intracellular or extracellular signals.

In most eukaryotic cells, the cell-cycle control system triggers cell-cycle progression at three major regulatory transitions, or checkpoints (*fig. 10.8*).

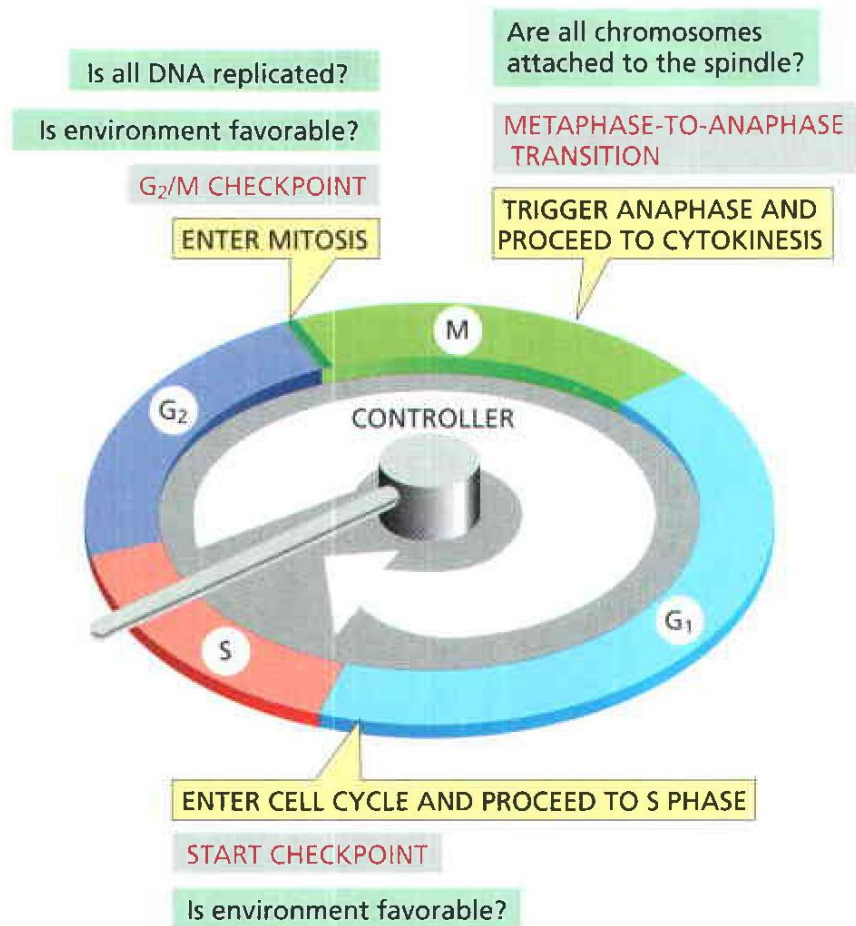


Figure 10.8. The control of the cell cycle (Alberts et al., 2008, p. 1061)

A cell-cycle control system triggers the essential processes of the cell cycle, such as DNA replication, mitosis, and cytokinesis. The control system is represented here as a central arm, the controller, that rotates clockwise, triggering essential processes when it reaches specific checkpoints on the outer dial. Information about the completion of cell-cycle events, as well as signals from the environment, can cause the control system to arrest the cycle at these checkpoints. The most prominent checkpoints occur at locations marked with yellow boxes

The first checkpoint is Start (or the restriction point) in late G₁, where the cell commits to cell-cycle entry and chromosome duplication, as mentioned earlier. The second is the G₂/M checkpoint, where the control system triggers the early mitotic events that lead to chromosome alignment on the spindle in metaphase. The third is the metaphase-to-anaphase transition, where the control system stimulates sister-chromatid separation, leading to the completion of mitosis and

cytokinesis. The control system blocks progression through each of these checkpoints if it detects problems inside or outside the cell. If the control system senses problems in the completion of DNA replication, for example, it will hold the cell at the G2/M checkpoint until those problems are solved. Similarly, if extracellular conditions are not appropriate for cell proliferation, the control system blocks progression through Start, thereby preventing cell division until conditions become favorable.

Central components of the cell-cycle control system are members of a family of protein kinases known as cyclin-dependent kinases (Cdks). The activities of these kinases rise and fall as the cell progresses through the cycle, leading to cyclical changes in the phosphorylation of intracellular proteins that initiate or regulate the major events of the cell cycle. An increase in Cdk activity at the G2/M checkpoint, for example, increases the phosphorylation of proteins that control chromosome condensation, nuclear envelope breakdown, spindle assembly, and other events that occur at the onset of mitosis.

Cyclical changes in Cdk activity are controlled by a complex array of enzymes and other proteins that regulate these kinases. The most important of these Cdk regulators are proteins known as cyclins. Cdks, as their name implies, are dependent on cyclins for their activity: unless they are tightly bound to a cyclin, they have no protein kinase activity (**fig. 10.9**).



Figure 10.9. Two key components of the cell-cycle control system (Alberts et al., 2008, p. 1062)

When cyclin forms a complex with Cdk, the protein kinase is activated to trigger specific cell-cycle events. Without cyclin, Cdk is inactive

Cyclins were originally named because they undergo a cycle of synthesis and degradation in each cell cycle. The levels of the Cdk proteins, by contrast, are constant, at least in the simplest cell cycles. Cyclical changes in cyclin protein levels result in the cyclic assembly and activation of the cyclin-Cdk complexes; this activation in turn triggers cell-cycle events.

There are four classes of cyclins, each defined by the stage of the cell cycle at which they bind Cdks and function. All eukaryotic cells require three of these classes:

1. G1/S-cyclins activate Cdks in late G1 and thereby help trigger progression through Start, resulting in a commitment to cell-cycle entry. Their levels fall in S phase.

2. S-cyclins bind Cdks soon after progression through Start and help stimulate chromosomes replication. S-cyclin levels remain elevated until mitosis, and these cyclins also contribute to the control of some early mitotic events.

3. M-cyclins activate Cdks that stimulate entry into mitosis at the G2/M checkpoint.

In most cells, a fourth class of cyclins, the G1-cyclins, helps govern the activities of the G1/S cyclins, which control progression through Start in late G1.

Most eukaryotic organisms reproduce sexually: the genomes of two parents mix to generate offspring that are genetically distinct from either parent. The cells of these organisms are generally diploid: that is, they contain two slightly different copies, or homologs, of each chromosome, one from each parent. Sexual reproduction depends on a specialized nuclear division process called meiosis, which produces haploid cells carrying only a single copy of each chromosome. In many organisms, the haploid cells differentiate into specialized reproductive cells called gametes: eggs and sperm in most species. In these species, the reproductive cycle ends when a sperm and egg fuse to form a diploid zygote with the potential to form a new individual. Here, we consider the basic mechanisms and regulation of meiosis, with an emphasis on how they compare with those of mitosis. Meiosis begins with a round of chromosome duplication, called meiotic S phase, followed by two rounds of chromosome segregation, called meiosis I and II. Meiosis I segregates the homologs (each composed of a tightly linked pair of sister chromatids). Meiosis II, like conventional mitosis, segregates the sister chromatids of each homolog (*fig. 10.10*).

The first meiotic division solves the central problem of meiosis: how to segregate the homologous chromosomes. Like sister-chromatid segregation in mitosis, homolog segregation in meiosis I depends on the formation of linkages between homologs. These linkages allow the homolog pairs to be bi-oriented on the first meiotic spindle, with the homologs in a pair attached to opposite poles. Homolog linkage is removed at the onset of anaphase I, allowing the spindle to pull the homologs to opposite ends of the cell.

Linkages form between homologs by a remarkably complex and lengthy process that occurs after meiotic S phase, in a period called meiotic prophase or prophase I. This process begins with homolog pairing, whereby the homologs gradually move close to each other in the nucleus, primarily as a result of interactions between complementary DNA sequences in the two homologs.

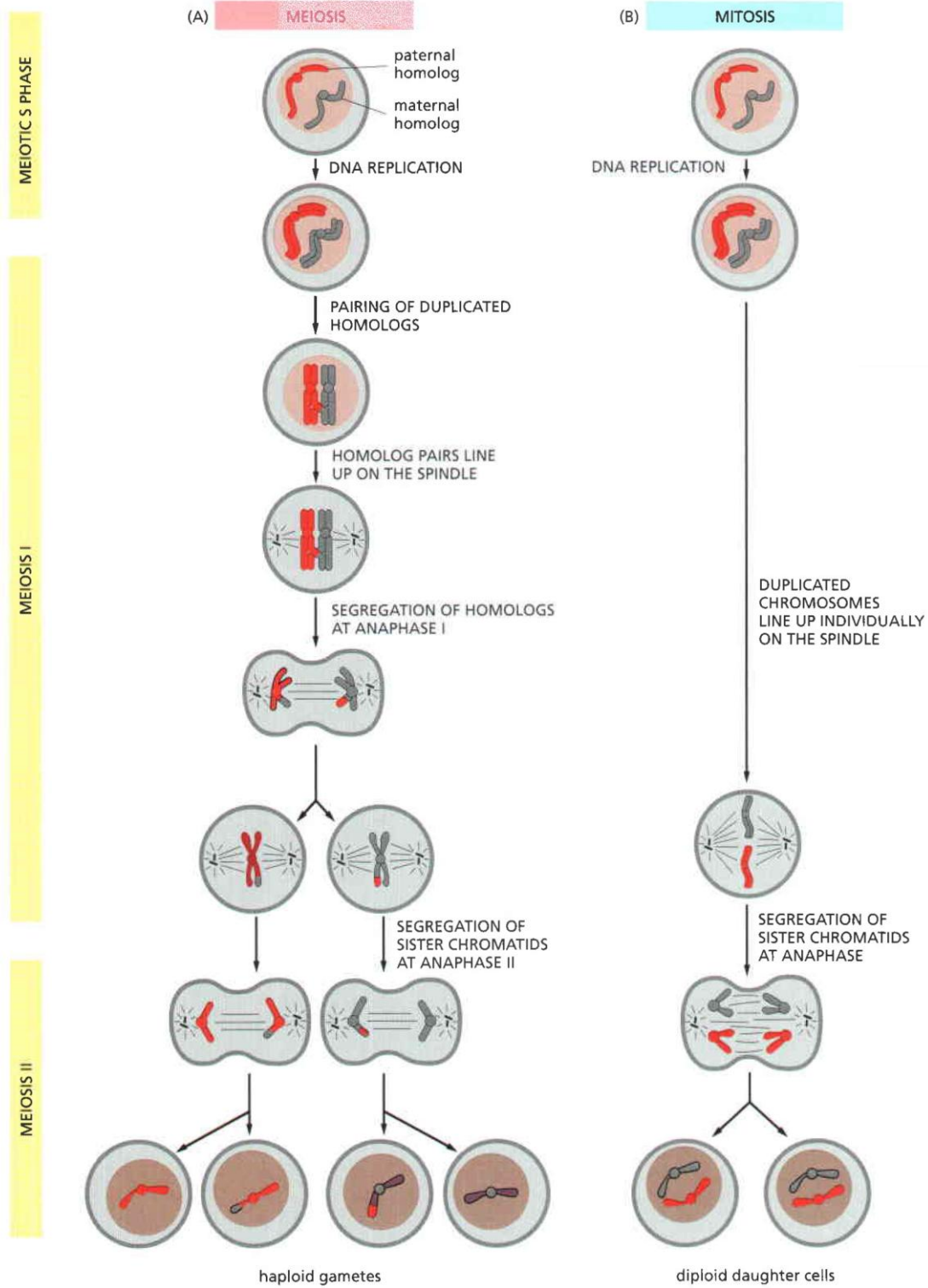


Figure 10.10. Comparison of meiosis and the mitotic cell cycle (Alberts et al., 2008, p. 1091)
 (A) Meiosis is a form of nuclear division in which a single round of chromosome duplication (meiotic S phase) is followed by two rounds of chromosome segregation. The first round, meiosis I, segregates the homologs, whereas the second round, meiosis II, segregates the sister chromatids. (B) In a mitotic cell cycle, by contrast, homologs do not pair up, and the sister chromatids segregate in a single division

Homolog linkages are then locked in place by homologous recombination between non-sister chromatids in each homolog pair: DNA double-strand breaks are formed at several locations in each sister chromatid, resulting in large numbers of DNA recombination events between the homologs. Some of these events lead to reciprocal DNA exchanges called crossovers, where the DNA of a chromatid crosses over to become continuous with the DNA of a homologous chromatid (**fig. 10.11**). At least one of these crossovers occurs in each homolog pair, ensuring that the homologs in every pair are physically connected when the cell enters the first meiotic division.

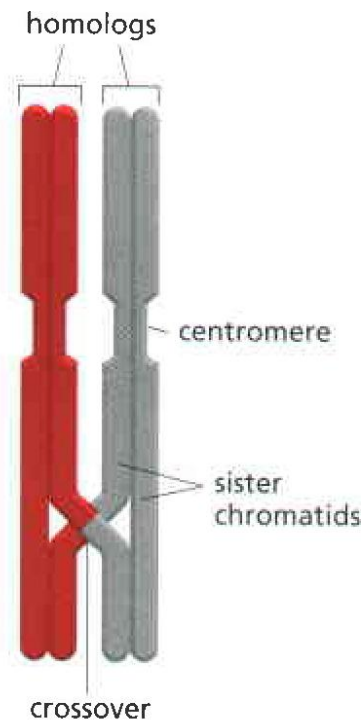


Figure 10.11. A crossover between homologs (Alberts et al., 2008, p. 1092)

As in mitosis, the sister chromatids in each homolog are tightly connected along their entire lengths. A single crossover has occurred between two non-sister chromatids in this example, but either of the two chromatids of a homolog can form a crossover with either chromatid of the other homolog, and it is usual for multiple crossovers to be formed

Each homolog contains two tightly linked sister chromatids, and thus the attachment of a homolog to a spindle pole requires that both sister kinetochores in a homolog attach to the same pole. This type of attachment is normally avoided during mitosis. Crossovers hold homolog pairs together only because the arms of the sister chromatids are connected by sister-chromatid cohesion. The loss of cohesion from sister-chromatid arms therefore triggers homolog separation at the onset of anaphase I. In contrast to mitosis, however, cohesin complexes near the centromeres remain uncleaved in meiosis I because cohesin in that region is protected from separase. Sister-chromatid pairs therefore remain linked at their centromeres throughout meiosis I, allowing their correct bi-orientation on the spindle in meiosis II. The mechanisms that block cohesin cleavage at the centromere in meiosis I are removed in meiosis II. At the onset of anaphase II, therefore triggers centromeric cohesin cleavage and sister chromatid separation – much as it does in mitosis.

CONTROL TESTS

Read attentively each assignment (question) and choose one correct answer from the choices offered below.

1. The cell cycle is:

- a) the period of cell life during the interphase;
- b) the period from the prophase to the interphase;
- c) the period from cell formation to its death;
- d) the period from cell formation in the process of division to its own division.

2. The period of the interphase during which DNA synthesis takes place is called:

- a) presynthetic period, G1 period;
- b) synthetic period;
- c) post-synthetic period.

3. What is the trigger causing the cells to enter the presynthetic cycle?

- a) biochemical growth factors;
- b) nerve stimulus;
- c) cell age.

4. Clusterosomes are:

- a) groups of replicons that form, together with replication enzymes, zones in the interphase nucleus where DNA synthesis takes place;
- b) cluster arrangement of genes in chromosomes;
- c) areas of highly spiralized chromatin.

TOPICS FOR INDEPENDENT WORK

Independent work includes the study of the following topics:

Topic 1: The life cycle of cells: presynthetic, synthetic, postsynthetic stages, mitosis. The significance of these phases in the cell life.

Topic 2: Structural and biochemical organization of the mitotic apparatus in eukaryotic cells.

Topic 3: The general scheme of mitosis in eukaryotic cells. The timeline of mitosis and cytokinesis.

Topic 4: Cytokinesis in animal and plant cells: cell constriction and phragmoplast formation. The biological meaning of mitosis.

Topic 5: Meiosis, meiotic stages. Conjugation of chromosomes, crossing over, the reduction of chromosome number. The biological meaning of meiosis.

Topic 6: The differences between mitosis and meiosis.

GLOSSARY

A

Amino acids are the basic building blocks of protein; there are 20 common amino acid types and their sequence will determine the properties and function of each protein.

Amitosis (karyostenosis) is cell proliferation that does not occur by mitosis, the mechanism usually identified as essential for cell division in eukaryotes.

Amplification is a mechanism leading to multiple copies of a chromosomal region within a chromosome arm. The DNA amplification technique of the polymerase chain reaction (PCR) in molecular biology, a laboratory method for creating multiple copies of small segments of DNA.

Anaphase is the stage of mitosis after the process of metaphase, when replicated chromosomes are split and the newly-copied chromosomes (daughter chromatids) are moved to opposite poles of the cell. Chromosomes also reach their overall maximum condensation in late anaphase, to help chromosome segregation and the re-formation of the nucleus.

Anaphase I is the stage of meiosis. Kinetochore microtubules shorten, pulling homologous chromosomes (which each consist of a pair of sister chromatids) to opposite poles. Nonkinetochore microtubules lengthen, pushing the centrosomes farther apart. The cell elongates in preparation for division down the center;

Antibody is an immunoglobulin molecule that reacts with a specific antigen that induced its synthesis. Synthesized by B lymphocytes that have been activated by the binding of an antigen to a cell surface receptor.

Anticodon is a unit made up of three nucleotides that correspond to the three bases of the codon on the mRNA. Each tRNA contains a distinct anticodon triplet sequence that can form 3 complementary base pairs to one or more codons for an amino acid.

Antigen is any substance, almost always a protein, not normally present in the body which when introduced to the body stimulates a specific immune response and the production of antibodies.

ATP is adenosine triphosphate. A chemical very important in energy metabolism in the cell.

Assimilation is the conversion of nutrient into the fluid or solid substance of the body, by the processes of digestion and absorption.

B

Bivalent is one pair of chromosomes (sister chromatids) in a tetrad. A tetrad is the association of two pairs of homologous chromosomes (4 sister chromatids)

physically held together by at least one DNA crossover. This physical attachment allows for alignment and segregation of the homologous chromosomes in the first meiotic division.

C

Camera lens is an optical lens or assembly of lenses used in conjunction with a camera body and mechanism to make images of objects either on photographic film or on other media capable of storing an image chemically or electronically.

Cell is the basic structural, functional, and biological unit of all known organisms. A cell is the smallest unit of life. Cells are often called the «building blocks of life». The study of cells is called cell biology, cellular biology, or cytology.

Cell membrane (also known as the **plasma membrane** or **cytoplasmic membrane**, and historically referred to as the **plasmalemma**) is a biological membrane that separates the interior of all cells from the outside environment (the extracellular space) which protects the cell from its environment. Cell membrane consists of a lipid bilayer, including cholesterol (a lipid component) that sit between phospholipids to maintain their fluidity under various temperature, in combination with membrane proteins such as integral proteins, and peripheral proteins.

Cell theory is the historic scientific theory, now universally accepted, that living organisms are made up of cells, that they are the basic structural/organizational unit of all organisms, and that all cells come from pre-existing cells. Cells are the basic unit of structure in all organisms and also the basic unit of reproduction. Cell theory was eventually formulated in 1839. This is usually credited to Matthias Schleiden and Theodor Schwann. However, many other scientists like Rudolf Virchow contributed to the theory.

Cell wall is a specialized form of extracellular matrix that surrounds every cell of a plant. The cell wall is made up of cellulose, hemicelluloses and pectin and is quite thick and rigid but generally permeable. Its major function is to provide protection and strength to the cell.

Centromere is the specialized DNA sequence of a chromosome that links a pair of sister chromatids (a dyad). During mitosis, spindle fibers attach to the centromere via the kinetochore. The physical role of the centromere is to act as the site of assembly of the kinetochores that is responsible for the actual events of chromosome segregation – i.e. binding microtubules.

Centrosome is a centrally located organelle of animal cells that is the primary microtubule-organizing center (MTOC) and acts as the spindle pole during mitosis. In most animal cells it contains a pair of centrioles.

Chiasma is the point where two chromatids are intertwined (interwoven) in a cell.

Chromatic aberration is caused by differences in refractive index for different wavelengths of light, in contrast with monochromatic aberration, which occurs for all frequencies of light.

Chromatid is a chromosome that has been newly copied or the copy of such a chromosome, the two of them still joined to the original chromosome by a single centromere. Before replication, one chromosome is composed of one DNA molecule. Following replication, each chromosome is composed of two DNA molecules; in other words, DNA replication itself increases the amount of DNA but does not (yet) increase the number of chromosomes. The two identical copies, each forming one half of the replicated chromosome are called chromatids. During the later stages of cell division these chromatids separate longitudinally to become individual chromosomes.

Chromatin is a complex of DNA and protein found in eukaryotic cells.

Chromomere is one of the serially aligned beads or granules of a eukaryotic chromosome, resulting from local coiling of a continuous DNA thread. In areas of chromatin with the absence of transcription, condensing of DNA and protein complexes will result in the formation of chromomeres.

Chromosome is a deoxyribonucleic acid (DNA) molecule with part or all of the genetic material (genome) of an organism. It is a structure composed of a very long DNA molecule and associated proteins (histones) that carries part (or all) of the hereditary information of an organism. Especially evident in plant and animal cells undergoing mitosis or meiosis, during which each chromosome becomes condensed into a compact rod like structure visible in the light microscope.

Crossing over is the exchange of genetic material between two non-sister chromatids takes place during meiosis cell division.

Cytokinesis is division of cytoplasm.

Cytology is the study of cells, their origin, structure, function and pathology; the microscopic examination of cells as a means of detecting malignancy and microbiologic changes. Cells can be obtained by aspiration, washing, smear or scraping.

Cytoplasm is all of the material within a cell, enclosed by the cell membrane, except for the cell nucleus. The inner layer of it is known as endoplasm and outer is known as cell cortex or ectoplasm. The material inside the nucleus is termed the nucleoplasm. The cytoplasm is about 80% water, consists of an aqueous substance cytosol in which variety of cell organelles and other inclusions like insoluble waste and storage products (starch, lipid etc.) are present.

Cytosine is one of the four main bases found in DNA and RNA, along with adenine, guanine, and thymine (uracil in RNA). It is a pyrimidine derivative. The nucleoside of cytosine is cytidine. In base pairing, it forms three hydrogen bonds with guanine.

Cytotoxin is a toxin or antibody having a specific toxic action upon the cells of special organs.

D

Defocus aberration occurs when a system is out of focus.

Deoxyribonucleic acid (DNA) is a molecule composed of two chains that coil around each other to form a double helix carrying genetic instructions for the development, functioning, growth and reproduction of all known organisms and many viruses. The two DNA strands are also known as polynucleotides as they are composed of simpler monomeric units called nucleotides.] Each nucleotide is composed of one of four nitrogen-containing nucleobases (cytosine [C], guanine [G], adenine [A] or thymine [T]), a sugar called deoxyribose, and a phosphate group. The nucleotides are joined to one another in a chain by covalent bonds between the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone. The nitrogenous bases of the two separate polynucleotide strands are bound together, according to base pairing rules (A with T and C with G), with hydrogen bonds to make double-stranded DNA. The complementary nitrogenous bases are divided into two groups, pyrimidines and purines. In DNA, the pyrimidines are thymine and cytosine; the purines are adenine and guanine.

Deoxyribose, or more precisely 2-deoxyribose is a monosaccharide a component of DNA.

Diakinesis is the stage of prophase I when chromosomes condense further. This is the first point in meiosis where the four parts of the tetrads are actually visible. Sites of crossing over entangle together, effectively overlapping, making chiasmata clearly visible. Other than this observation, the rest of the stage closely resembles prometaphase of mitosis; the nucleoli disappear, the nuclear membrane disintegrates into vesicles, and the meiotic spindle begins to form.

Diploid refers to cells having a pair of each type of chromosome. Most somatic cells of higher organisms are diploid.

Diplotene, also known as **diplonema**, from Greek words meaning "two threads", is the stage of meiosis when the synaptonemal complex degrades and homologous chromosomes separate from one another a little. The chromosomes themselves uncoil a bit, allowing some transcription of DNA. However, the homologous chromosomes of each bivalent remain tightly bound at chiasmata, the regions where crossing-over occurred. The chiasmata remain on the chromosomes until they are severed at the transition to anaphase I.

DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule. DNA replication occurs in all living organisms acting as the basis for biological inheritance. The cell possesses the distinctive property of division, which makes replication of DNA essential. DNA is made up of a double helix of two complementary strands. During replication, these strands are separated. Each strand of the original DNA molecule then serves as a template for the production of its counterpart, a process referred to as semiconservative replication. As a result of semi-conservative replication, the new helix will be composed of an original DNA strand as well as a newly synthesized

strand. Cellular proofreading and error-checking mechanisms ensure near perfect fidelity for DNA replication. In a cell, DNA replication begins at specific locations, or origins of replication, in the genome. Unwinding of DNA at the origin and synthesis of new strands, accommodated by an enzyme known as helicase, results in replication forks growing bi-directionally from the origin. A number of proteins are associated with the replication fork to help in the initiation and continuation of DNA synthesis. Most prominently, DNA polymerase synthesizes the new strands by adding nucleotides that complement each (template) strand. DNA replication occurs during the S-stage of interphase.

E

Endoplasmic reticulum (ER) is a membranous network inside the cell enclosing a fluid filled lumen which almost filled the intracellular cavity. It is of two types: rough endoplasmic reticulum (RER): with ribosomes attached on its surface for synthesizing proteins, and smooth endoplasmic reticulum (SER): which is without ribosomes and is meant for secreting lipids. ER forms supporting skeletal framework of the cell and also provides a pathway for distribution of nuclear material from one cell to another.

Enzymes are both proteins and biological catalysts (biocatalysts). Catalysts accelerate chemical reactions. The molecules upon which enzymes may act are called substrates, and the enzyme converts the substrates into different molecules known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life.

Eosin is any of a class of rose-colored stains or dyes; bromine derivatives of fluorescein; used in histology as a stain.

G

Gamete is a haploid cell that fuses with another haploid cell during fertilization in organisms that sexually reproduce.

Gene is a sequence of nucleotides in DNA or RNA that encodes the synthesis of a gene product, either RNA or protein.

Genetic code is the set of rules used by living cells to translate information encoded within genetic material (DNA or mRNA sequences of nucleotide triplets, or codons) into proteins. Translation is accomplished by the ribosome, which links amino acids in an order specified by messenger RNA (mRNA), using transfer RNA (tRNA) molecules to carry amino acids and to read the mRNA three nucleotides at a time. The genetic code is highly similar among all organisms and can be expressed in a simple table with 64 entries.

Genotype is the genetic constitution of an organism.

Glycocalyx, or pericellular matrix, is a glycoprotein and glycolipid covering that surrounds the cell membranes of some bacteria, epithelia, and other cells.

Golgi apparatus consists of a set of membrane-bounded, fluid-filled vesicles, vacuoles and flattened cisternae (closed sacs). Its main function is secretory. It packages material synthesised inside the cell and dispatches them. It produces vacuoles or secretory vesicles which contain cellular secretions like enzymes etc. It is also involved in the secretion of cell wall, plasma membrane and lysosomes.

Guanine (G, Gua) is one of the four main nucleobases found in the nucleic acids DNA and RNA, the others being adenine, cytosine, and thymine (uracil in RNA). In DNA, guanine is paired with cytosine. The guanine nucleoside is called guanosine.

H

Haploid is the quality of a cell or organism having a single set of chromosomes. Organisms that reproduce asexually are haploid. Sexually reproducing organisms are diploid (having two sets of chromosomes, one from each parent). In humans, only their egg and sperm cells are haploid.

Heterochromatin is a tightly packed form of DNA or condensed DNA, which comes in multiple varieties. These varieties lie on a continuum between the two extremes of constitutive heterochromatin and facultative heterochromatin. Both play a role in the expression of genes.

Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes.

Homologous chromosomes or homologs are a set of one maternal and one paternal chromosome that pair up with each other inside a cell during fertilization. Homologs have the same genes in the same loci where they provide points along each chromosome which enable a pair of chromosomes to align correctly with each other before separating during meiosis.

I

Immersion microscopy is an optical technique used to increase the resolving power of a microscope by immersing both the objective lens and the specimen in a transparent oil, thereby increasing the numerical aperture of the objective lens.

Interphase is the phase of the cell cycle in which a typical cell spends most of its life. Prophase, however is the longest stage of mitosis. During interphase, the cell copies its DNA in preparation for mitosis. Interphase is the 'daily living' or metabolic phase of the cell, in which the cell obtains nutrients and metabolizes them, grows, reads its DNA, and conducts other «normal» cell functions.

K

Karyokinesis is the division of nucleus during cell division.

Kinetochores are disc-shaped protein structures associated with duplicated chromatids in eukaryotic cells where the spindle fibers attach during cell division.

to pull sister chromatids apart. The kinetochore assembles on the centromere and links the chromosome to microtubule polymers from the mitotic spindle during mitosis and meiosis. Its proteins also help to hold the sister chromatids together and play a role in chromosome editing.

L

Lateral: situated away from the midline of the body.

Leptotene is the first stage of prophase I. It is also known as leptonema, from Greek words meaning «thin threads». In this stage of prophase I, individual chromosomes—each consisting of two sister chromatids—become «individualized» to form visible strands within the nucleus. The two sister chromatids closely associate and are visually indistinguishable from one another. During leptotene, lateral elements of the synaptonemal complex assemble. Leptotene is of very short duration and progressive condensation and coiling of chromosome fibers takes place.

Leukocytes are white blood cells made in the bone marrow and found in the blood and lymph tissue. Leukocytes are part of the body's immune system.

Leukocytosis is a transient increase in the number of white blood cells (leukocytes); due to various causes.

Lysosomes are simply tiny spherical sac-like structures evenly distributed in the cytoplasm. Its cells digest foreign proteins, bacteria and viruses. So, it is a kind of garbage disposable system of the cell. They are also known as suicidal bags as when the cells get damaged, lysosomes may burst and enzymes eat up their own cells.

M

Medial: situated closer to the midline of the body.

Meiosis is a special type of cell division used by sexually-reproducing organisms to produce the gametes, such as sperm or egg cells. It involves two rounds of division that ultimately result in four cells with only one copy of each chromosome (haploid). Additionally, prior to the division, genetic material from the paternal and maternal copies of each chromosome is crossed over, creating new combinations of code on each chromosome.

Metaphase I is the stage of meiosis when homologous pairs move together along the metaphase plate. As kinetochore microtubules from both centrosomes attach to their respective kinetochores, the paired homologous chromosomes align along an equatorial plane that bisects the spindle, due to continuous counterbalancing forces exerted on the bivalents by the microtubules emanating from the two kinetochores of homologous chromosomes.

Mitochondria are tiny bodies of varying shapes and size, distributed in the cytoplasm and bounded by a double membrane envelope. The outer membrane is porous and the inner membrane is thrown in to folds known as cristae having some

rounded bodies known as F1 particles or oxysomes. Since mitochondria synthesize energy rich compounds (ATP), they are known as the power house of the cell.

N

Nitrogenous base (nitrogen-containing base) is an organic molecule with a nitrogen atom that has the chemical properties of a base. The main biological function of a nitrogenous base is to bond nucleic acids together. A nitrogenous base owes its basic properties to the lone pair of electrons of a nitrogen atom. Nitrogenous bases are typically classified as the derivatives of two parent compounds, pyrimidine and purine. They are non-polar and due to their aromaticity, planar. Both pyrimidines and purines resemble pyridine and are thus weak bases and relatively unreactive towards electrophilic aromatic substitution.

Nucleolus is the structure in the nucleus of eukaryotic cells. It is best known as the site of ribosome biogenesis. Nucleoli are made of proteins, DNA and RNA and form around specific chromosomal regions called nucleolar organizing regions.

Nucleus is a spherical cellular component, centrally located in the cell and filled with a fluid, namely cytoplasm. It is bounded by two nuclear membranes forming a nuclear envelope. The space between nuclear envelope is connected to Endoplasmic reticulum (ER). It also separates the nucleus from the cytoplasm and its pores contain the liquid known as nucleoplasm which is embedded with two structures – the nucleolus and chromatin material. It is rich in protein and RNA (ribonucleic acid) and is also known as the factory of ribosomes because of the ribosome formation.

O

Operon is a functioning unit of DNA containing a cluster of genes under the control of a single promoter. The genes are transcribed together into an mRNA strand and either translated together in the cytoplasm, or undergo splicing to create monocistronic mRNAs that are translated separately, i.e. several strands of mRNA that each encode a single gene product. The result of this is that the genes contained in the operon are either expressed together or not at all.

Optical aberration is an imperfection in image formation by an optical system.

P

Pachytene is the stage meiosis, also known as pachynema, at this point a tetrad of the chromosomes has formed known as a bivalent. This is the stage when homologous recombination, including chromosomal crossover (crossing over), occurs. Nonsister chromatids of homologous chromosomes may exchange genetic information over regions of homology.

Peptide is a protein with a small number of amino acids.

Peroxisomes are small and spherical organelles containing powerful oxidative enzymes. They carry out some oxidative reactions like detoxification or removal of toxic substances from the cell.

Phenotype is a set of all the observable characteristics of an organism that result from the interaction of its genotype (total genetic inheritance) with the environment. Examples of observable characteristics include behaviour, biochemical properties, colour, shape, and size.

Phragmoplast is a plant cell specific structure that forms during late cytokinesis. The phragmoplast is a complex assembly of microtubules (MTs), microfilaments (MFs), and endoplasmic reticulum (ER) elements, that assemble in two opposing sets perpendicular to the plane of the future cell plate during anaphase and telophase. It is initially barrel-shaped and forms from the mitotic spindle between the two daughter nuclei while nuclear envelopes reassemble around them. The cell plate initially forms as a disc between the two halves of the phragmoplast structure.

Plasma membrane is the outer lining of the cell. This thin, flexible and selectively permeable membrane separates the cell from its environment and allows materials to enter and leave the cell. It is made up of lipids, proteins and a small number of carbohydrates.

Plastids occur in plant cell and are absent in animal cell. They have their own genome and have power to divide. They are of three types: Chromoplasts (coloured plastids) which impart various colours to flowers to attract insects for pollination. Chloroplasts (green-coloured plastids) trap solar energy and utilise it to manufacture food for the plant. Leucoplasts (colourless plastids) store food in the form of carbohydrates (starch), fats and proteins.

Polytene chromosomes are large chromosomes which have thousands of DNA strands. Polytene chromosomes were first reported by E.G. Balbiani in 1881. Polytene chromosomes are found in dipteran flies: the best understood are those of *Drosophila*, *Chironomus* and *Rhynchosciara*. They are produced when repeated rounds of DNA replication without cell division forms a giant chromosome. Thus, polytene chromosomes form when multiple rounds of replication produce many sister chromatids which stay fused together.

Post-transcriptional modification or co-transcriptional modification is a set of biological processes common to most eukaryotic cells by which an RNA primary transcript is chemically altered following transcription from a gene to produce a mature, functional RNA molecule that can then leave the nucleus and perform any of a variety of different functions in the cell. There are many types of post-transcriptional modifications achieved through a diverse class of molecular mechanisms.

Prokaryote is a unicellular organism that lacks a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle. Prokaryotes are divided into two domains, Archaea and Bacteria. Prokaryotes are asexual, reproducing without fusion of gametes. The first living organisms are thought to have been prokaryotes.

Prophase is the first stage of cell division in both mitosis and meiosis. It begins after interphase. DNA has already been replicated when the cell enters prophase. The main occurrences in prophase are the condensation of the chromatin and the disappearance of the nucleolus.

R

Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes. RNA and DNA are nucleic acids, and, along with lipids, proteins and carbohydrates, constitute the four major macromolecules essential for all known forms of life. Like DNA, RNA is assembled as a chain of nucleotides, but unlike DNA, RNA is found in nature as a single strand folded onto itself, rather than a paired double strand. Cellular organisms use messenger RNA (mRNA) to convey genetic information (using the nitrogenous bases of guanine, uracil, adenine, and cytosine, denoted by the letters G, U, A, and C) that directs synthesis of specific proteins. Many viruses encode their genetic information using an RNA genome.

Ribose is a simple sugar and carbohydrate with molecular formula $C_5H_{10}O_5$. The naturally-occurring form, d-ribose, is a component of the ribonucleotides from which RNA is built, and so this compound is necessary for coding, decoding, regulation and expression of genes.

Ribosomes are dense, spherical and granular particles which occur freely in the matrix (cytosol) or remain attached to the ER. Ribosomes play an important part in the synthesis of proteins.

RNA polymerase (ribonucleic acid polymerase, RNAP or RNAPol, officially DNA-directed RNA polymerase) is an enzyme that synthesizes RNA from a DNA template. RNAP locally opens the double-stranded DNA (usually about four turns of the double helix) so that one strand of the exposed nucleotides can be used as a template for the synthesis of RNA, a process called transcription. A transcription factor and its associated transcription mediator complex must be attached to a DNA binding site called a promoter region before RNAP can initiate the DNA unwinding at that position. RNAP not only initiates RNA transcription, it also guides the nucleotides into position, facilitates attachment and elongation, has intrinsic proofreading and replacement capabilities, and termination recognition capability.

RNA splicing is a form of RNA processing in which a newly made precursor messenger RNA (pre-mRNA) transcript is transformed into a mature messenger RNA (mRNA). During splicing, introns (Non-coding regions) are removed and exons (Coding Regions) are joined together. For nuclear-encoded genes, splicing takes place within the nucleus either during or immediately after transcription. For those eukaryotic genes that contain introns, splicing is usually required in order to create an mRNA molecule that can be translated into protein.

S

Spherical aberration occurs when light rays pass through a spherical lens near the edge.

Spherosomes or oleosomes are small cell organelles bounded by single membrane which take part in storage and synthesis of lipids. They arise from smooth endoplasmic reticulum.

Spindle apparatus (or mitotic spindle) refers to the cytoskeletal structure of eukaryotic cells that forms during cell division to separate sister chromatids between daughter cells. It is referred to as the mitotic spindle during mitosis, a process that produces genetically identical daughter cells, or the meiotic spindle during meiosis, a process that produces gametes with half the number of chromosomes of the parent cell.

Stroma is the connective tissue framework of an organ or other structure, as distinguished from the tissues performing the special function of the organ.

Synapsis (also called syndesis) is the pairing of two homologous chromosomes that occurs during meiosis. It allows matching-up of homologous pairs prior to their segregation, and possible chromosomal crossover between them. Synapsis takes place during prophase I of meiosis.

Synchronous processes of meiosis: during these stages, two centrosomes, containing a pair of centrioles in animal cells, migrate to the two poles of the cell. These centrosomes, which were duplicated during S-phase, function as microtubule organizing centers nucleating microtubules, which are essentially cellular ropes and poles. The microtubules invade the nuclear region after the nuclear envelope disintegrates, attaching to the chromosomes at the kinetochore. The kinetochore functions as a motor, pulling the chromosome along the attached microtubule toward the originating centrosome, like a train on a track. There are four kinetochores on each tetrad, but the pair of kinetochores on each sister chromatid fuses and functions as a unit during meiosis

T

Telomere is a region of repetitive nucleotide sequences at each end of a chromosome, which protects the end of the chromosome from deterioration or from fusion with neighboring chromosomes.

Telophase is the final stage in both meiosis and mitosis in a eukaryotic cell. As chromosomes reach the cell poles, a nuclear envelope is re-assembled around each set of chromatids, the nucleoli reappear, and chromosomes begin to decondense back into the expanded chromatin that is present during interphase. The mitotic spindle is disassembled and remaining spindle microtubules are depolymerized. Telophase accounts for approximately 2% of the cell cycle's duration.

Telophase I is the stage of meiosis. The first meiotic division effectively ends when the chromosomes arrive at the poles. The microtubules that make up the

spindle network disappear, and a new nuclear membrane surrounds each haploid set.

Thymine (T, Thy) is one of the four nucleobases in the nucleic acid of DNA. Thymine is also known as 5-methyluracil, a pyrimidine nucleobase. In RNA, thymine is replaced by the nucleobase uracil.

Tonoplast is the membrane surrounding the vacuole in a plant cell.

Transcription is the first of several steps of DNA based gene expression (a gene is a fragment of DNA that encodes for a protein, some genes encode for a 'non-coding' mRNA such as microRNA or long non-coding RNA), in which a particular segment of DNA is copied into RNA (especially mRNA) by the enzyme RNA polymerase. During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand called a primary transcript.

Translation is the process in which ribosomes in the cytoplasm or ER synthesize proteins after the process of transcription of DNA to RNA in the cell's nucleus. The entire process is called gene expression.

U

Unit membrane is the basic structure of cell membrane.

Uracil is one of the four nucleobases in the nucleic acid of RNA. The others are adenine (A), cytosine (C), and guanine (G). In RNA, uracil binds to adenine via two hydrogen bonds. In DNA, the uracil nucleobase is replaced by thymine. Uracil is a common and naturally occurring pyrimidine derivative.

V

Vacuole is a membrane-bound organelle which is present in all plant and fungal cells and some protist, animal and bacterial cells. Vacuoles are essentially enclosed compartments which are filled with water containing inorganic and organic molecules including enzymes in solution, though in certain cases they may contain solids which have been engulfed. Vacuoles are formed by the fusion of multiple membrane vesicles and are effectively just larger forms of these. The organelle has no basic shape or size; its structure varies according to the requirements of the cell.

Z

Zygotene is the stage of meiosis, also known as zygonema, it occurs as the chromosomes approximately line up with each other into homologous chromosome pairs. In some organisms, this is called the bouquet stage because of the way the telomeres cluster at one end of the nucleus.

INFORMATION RESOURCES FOR THE SUBJECT «CYTOLOGY»

a) main course literature

1. Alberts B. Molecular Biology of the Cell. 2015. WW Norton & Co, CT, United States ISBN10 0815344643; ISBN13 9780815344643. URL: https://www.bookdepository.com/Molecular-Biology-Cell-Bruce-Alberts/9780815344643?ref=pd_detail_2_sims_cat_bs_1
2. Alberts B., Johnson A., Lewis J. et al. Molecular biology of the cell. Garland Science, 2008, 1725 p.
3. Molecular Biology. Springer Science+Business Media New York 2014/ Ed. S.H. Howell. ISBN 978-1-4614-7569-9; ISBN 978-1-4614-7570-5 / URL: <https://link.springer.com/referencework/10.1007/978-1-4939-0263-7>
4. Molecular Cell Biology. 2016.1280 p. Ed. H. Lodish, A. Berk , C.A. Kaiser, A. Amon et al. ISBN10 1464187444; ISBN13 9781464187445. URL: https://www.bookdepository.com/Molecular-Cell-Biology-Harvey-Lodish/9781464187445?ref=pd_detail_1_sims_b_p2p_1
5. Клетки / Под ред Б. Льюина и др. – М.: Бином. Лаборатория знаний, 2011. – 951 с.
6. Ченцов, Ю.С. Введение в клеточную биологию. / Ю.С. Ченцов. – М.: Альянс, 2015 – 495 с.

b) further reading

1. Cell Structure and Function. Oxford University Press, 2014. 520 p. ISBN13 9780199652471; ISBN10 0199652473; URL: <https://www.bookdepository.com/Cell-Structure-Function-Guy-Orchard/9780199652471?ref=grid-view>
2. Eukaryotic Membranes and Cytoskeleton. Origins and Evolution, Advances in Experimental Medicine and Biology (2007) / URL: <https://link.springer.com/book/10.1007/978-0-387-74021-8>
3. Lysosomes Paul Saftig Ph.D. in Medical Intelligence Unit (2005) / URL: <https://link.springer.com/book/10.1007/0-387-28957-7>
4. Mitochondria in Health and in Sickness Prof. Andrea Urbani, Dr. Mohan Babu in Advances in Experimental Medicine and Biology (2019) / URL: <https://link.springer.com/book/10.1007/978-981-13-8367-0>
5. Афанасьев, Ю.И. Гистология, эмбриология, цитология: учебник / Ю.И. Афанасьев, Н.А. Юрина, Б.В. Алешин. – М.: ГЭОТАР-Медиа, 2016. – 800 с.
6. Кузнецов, С.Л. Гистология, цитология, эмбриология: Учебник / С.Л. Кузнецов, Н.Н. Мушкамбаров. 3-е изд., испр. и доп. – Медицинское информационное агентство, 2016. – 180 с.

7. Романова Е.Б. Основы клеточной биологии / Электронный управляемый курс на сайте электронного обучения ННГУ им. Н.И. Лобачевского, в среде Moodle. – 2016. Идентификационный номер: 1333У.01.16 // URL: <http://e-learning.unn.ru/course/view.php?id=1333>.

8. Романова Е.Б. Основы цитологии. Электронный управляемый курс (ЭУК), 2019. Идентификационный номер: 62Е.01.2019 // URL: <https://mooc.unn.ru/course/view.php?id=62>

9. Слюсарев, А.А. Биология с общей генетикой: учебник / А.А. Слюсарев. – М.: Альянс, 2015. – 472с.

c) software and Internet resources

1. Encyclopedia Britannica, 2010 [Digital resource]. URL: <http://www.britannica.com/bps/media-view/114953/1/0/0>

2. Wikimedia Foundation, Inc. [Digital resource]. URL: <http://upload.wikimedia.org/wikipedia/commons/thumb/2/26/Chloroplast.svg/2000px-Chloroplast.svg.png>

3. Atlas, Loyola University Medical Education Network (Chicago, USA). URL:

http://www.meddean.luc.edu/lumen/MedEd/Histo/frames/histo_frames.html

4. A small educational program containing a set of animated illustrations on animal and plant cell cytophysiology. URL: <http://www.cellsalive.com/>

5. An educational program of the University of Arizona (USA) containing detailed text information and illustrations on history, methods of cell research, cell life cycle (including mitosis), cytoskeleton. Each section is provided with self-control tools (multiple choice tests). URL: http://www.biology.arizona.edu/cell_bio/cell_bio.html

Elena Romanova

CYTOLOGY

Manual

Russian text translated by A.G. Lubavsky

Подписано к печати 29.07.20. Формат 60×84/16.

Уч.-изд. л. 7,9. Усл. печ. л. 7,44.

Заказ № 190. Тираж 300.

Lobachevsky State University of Nizhny Novgorod

603950, Nizhny Novgorod, Gagarin Ave., 23

Printed at the printing shop of the Lobachevsky State University
of Nizhny Novgorod

603000, Nizhny Novgorod, 37 Bolshaya Pokrovskaya St.