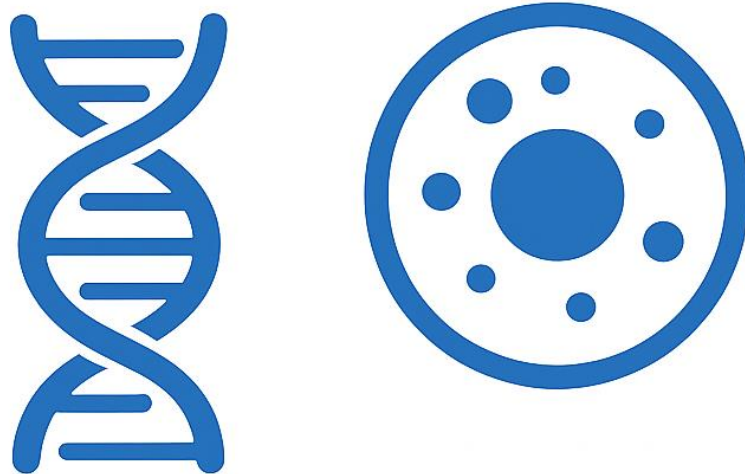


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Daniela Klimová
and colleagues



Medical Biology and Genetics 1

Repetitorium and Model Questions



UNIVERZITA
KOMENSKÉHO
V BRATISLAVE

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**MEDICAL BIOLOGY AND GENETICS 1
REPETITORIUM AND MODEL QUESTIONS**

University Textbook for Undergraduate Medical Education

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Preface

Dear Students,

You are holding a resource designed to guide you through the study of medical biology and genetics. These fields form the foundation of medicine, and understanding them is essential for navigating other subjects as well as clinical practice.

In this text, we have prepared the key topics of the first semester and supplemented them with a set of model questions. These questions are divided into 14 chapters corresponding to the content of lectures and practical sessions. They are intended to consolidate your knowledge; however, they do not replace the prescribed study literature. At the end of each chapter, you will find an answer key that will provide you with immediate feedback and support for self-assessment.

Our aim was to create material that would help you not only in exam preparation but also in gaining a deeper understanding of the fundamentals of biology and genetics. We believe it will enable you to study more systematically, review important facts, and approach final evaluations with greater confidence.

We sincerely wish you much success in your studies.

Štefan Harsányi, Daniela Klimová and colleagues

1 Introduction to microscopy techniques

Microscopes are among the most fundamental instruments in modern science and technology, particularly in the biomedical fields. Their primary function is to enable the observation of objects too small for the human eye to detect (smaller than approximately 70 μm). This includes various biological specimens such as cells, bacteria, and other microorganisms. Microscopes have enabled key discoveries in biology, medicine, and numerous other fields.

Types of Microscopes

From the earliest simple devices, microscopes have undergone numerous improvements, culminating in the development of modern light and electron microscopes. The **light microscope**, the most commonly used, employs visible or ultraviolet light as its source of illumination. Its optical components are typically made of precision-ground glass, ensuring high accuracy and resolving power. The **resolution limit** of the light microscope is approximately 0.2 micrometers (μm), meaning it can distinguish two points separated by that distance. The theoretical maximum magnification of a light microscope is approximately 2000 $\times$, although magnifications of around 1000 $\times$ are most commonly used in practice.

To observe finer details beyond the resolving capacity of light microscopes, **electron microscopes** were developed. These use a beam of electrons emitted by a cathode and focused with specialized electromagnetic lenses. As a result, they achieve far higher resolution than light microscopes (up to 0.2 nm) and can reach magnifications of up to one million times. Electron microscopes are divided into two main types: **transmission electron microscopes (TEM)** and **scanning electron microscopes (SEM)**. TEM directs the electron beam through an ultrathin specimen, producing highly detailed images of internal structures. In contrast, SEM scans the specimen's surface and detects (records) secondary or backscattered electrons, thereby generating a three-dimensional image of surface features.

Light microscopes are further classified into several specialized types according to their applications. These include the **stereomicroscope**, inverted microscope, fluorescence microscope, and polarization microscope. The stereomicroscope provides a three-dimensional image of the observed object, which is especially useful in diagnostics or during surgical procedures such as gynecological, neurosurgical, and ophthalmological operations. Although it does not achieve very high magnifications, it provides a sufficient view of the observed structure or surgical field. Stereomicroscopes use two separate optical paths (systems), which allows for the creation of a stereoscopic image of an object.

The **inverted microscope** is another specialized light microscope, primarily used for observing cell and tissue cultures. Unlike the conventional light microscope, the inverted version places the optical system below the specimen and the illumination source above it. This arrangement allows the observation of cells growing at the bottom of culture vessels.

The **fluorescence microscope** is a specialized form of light microscope. Traditional fluorescence microscopes typically used mercury or xenon discharge lamps as the light source, whereas modern instruments increasingly employ LED systems, and lasers dominate in confocal and super-resolution microscopy. Light from the source passes through an excitation filter, transmitting only the wavelengths required to excite fluorophores. After absorbing this energy, the fluorophores shift into an excited state and then release part of the energy as photons of longer wavelength, known as emission. The emitted light is filtered by an emission filter and directed to the eyepiece or camera. This method enables the visualization of cellular and tissue structures that are otherwise difficult to detect, making fluorescence microscopy indispensable in modern molecular cytogenetics. It is widely used in techniques such as fluorescence in situ hybridization (FISH), which enables the direct identification of genetic alterations within cells.

Conventional fluorescence microscopy is limited in resolution by the diffraction limit of light. This limitation is partially overcome by **confocal microscopy**, which uses a laser light source focused on a precisely defined point. Light originating from the same focal plane passes through a pinhole aperture, thereby eliminating out-of-focus signals from other layers of the specimen. This results in enhanced contrast, improved spatial resolution, and the ability to obtain optical sections of thicker samples, which can subsequently be reconstructed into a three-dimensional image. Pushing further, super-resolution microscopy (e.g., STED, PALM, STORM) surpasses the diffraction limit altogether, achieving resolutions of 20–50 nm. These methods employ specialized optical principles, such as suppressing fluorescence outside a narrowly defined spot or the sequential localization of individual molecules. Such techniques enable the investigation of subcellular structures and dynamic processes that were previously accessible only through electron microscopy.

The **polarization microscope** is another specialized light microscope that employs polarized light. It is used to study structures with birefringent or anisotropic properties, such as crystalline inclusions or fibers. In biomedicine, polarization microscopy is often used to observe specific tissues and cellular structures that exhibit these characteristics.

Design of Light Microscopes

The light microscope consists of three main components: the optical, illumination, and mechanical systems. The **optical system** includes the objective and the eyepiece. The objective is a set of lenses positioned above the specimen that creates a magnified intermediate image. The eyepiece is the lens through which this image is viewed and further magnified. The **illumination system** provides adequate lighting of the specimen; the light source is typically a halogen or LED bulb. The light passes through optical elements such as lenses, condensers, and filters, which ensure even and optimal illumination of the specimen.

The **mechanical system** of the microscope comprises the body tube, which maintains the correct distance between the objective and the eyepiece, and the stage, where the specimen is securely positioned. Focusing is achieved by moving the body tube or stage with a coarse

adjustment knob (macroscrew) for rough focus and a fine adjustment knob (microscrew) for precise focus. The stage is equipped with a mechanism that allows accurate horizontal movement of the specimen in the anteroposterior and lateral directions.

Practical Aspects of Working with a Microscope

When working with a microscope, it is essential to follow basic principles of proper handling, maintenance, and safety. Adhering to these rules ensures not only the long lifespan of the instrument but also the accuracy and reproducibility of observations.

The optical components of the microscope are very sensitive and must be handled with care. Objectives and eyepieces should be cleaned only with specialized wipes or solutions designed for optical surfaces. When not in use for extended periods, the microscope should be covered with a protective case to prevent dust accumulation. It should be stored in a stable environment, free of excessive humidity and vibration.

Accurate measurement of microscopic structures requires calibration with an ocular micrometer and a standard reference slide. Calibration allows precise determination of the size of cells, organelles, or other microstructures at a given magnification. This procedure is particularly important in histology, cytology, and microbiology, where measurement accuracy directly influences the diagnostic value of observations.

Specimens observed under the microscope may be native or fixed. **Native preparations (wet mounts)** are examined without prior processing; for example, a drop of blood, urine, a swab, or cultured cells may be placed directly on a glass slide and covered with a coverslip. Their advantage is the possibility of observing living cells and dynamic processes, such as flagellar movement or phagocytosis; their disadvantage is low contrast and limited stability. **Fixed specimens**, in contrast, are more permanent and allow more detailed study. The material is preserved either by heat or chemically (e.g., with formalin or alcohol) to maintain cellular and tissue structures, and then stained to highlight internal details. Fixed specimens form the basis of histological and cytological examinations.

Microscopic specimens are often transparent, and without staining, they would exhibit little contrast, making it difficult to observe structural details. **Staining** enhances cellular and tissue structures, allowing for the differentiation of cell types or microorganisms. Basic methods include simple staining (e.g., methylene blue), differential staining (e.g., Gram staining of bacteria), and special stains (e.g., for lipids, connective tissue, or nucleic acids). Giemsa stain is a specialized cytological method. It provides a detailed visualization of nuclei, cytoplasm, and parasites. It is used particularly in hematology for staining blood smears (differential blood count) and in cytogenetics for G-banding of chromosomes.

When working with light sources such as mercury lamps, UV sources, or lasers, it is necessary to follow principles of eye and skin protection. Protective goggles with suitable filters must be used, and direct exposure to the beam must be avoided. Proper ventilation of the workplace is

also crucial in preventing exposure to harmful vapors emitted by certain older light sources. The following table provides an overview of the fundamental concepts used in microscopy.

Term	Definition	Practical Significance
Resolution	The minimum distance at which two points can still be distinguished as separate.	Determines the level of detail observable; more important than magnification alone.
Magnification	The ratio of the image size to the actual size of the object.	Without adequate resolution, high magnification does not yield a clear image.
Numerical Aperture (NA)	A dimensionless value expressing the objective's ability to gather light; it depends on the angle of light rays and the refractive index of the medium.	Higher NA = more light collection, improved contrast, and better resolution.
Immersion Medium	Oil or water is placed between the coverslip and the front lens of the objective.	Increases NA, enhancing sharpness and contrast at high magnifications (especially with 100× objectives).
Spherical Aberration	Differential refraction of light through the center versus the periphery of a lens.	Produces a blurred image; corrected with specially designed lenses.
Chromatic Aberration	Differential refraction of light of different wavelengths.	Creates colored fringes at edges; corrected by achromatic or apochromatic objectives.
Astigmatism	Light rays in different planes fail to converge at a single focal point.	Image appears sharp in only one direction; corrected with specialized lens designs.



Model questions

1. What type of radiation is used in fluorescence microscopy?

- a) Infrared radiation
- b) Visible excitation light
- c) Ultraviolet radiation
- d) Electron beam

2. Which stain is most commonly used for chromosome visualization?

- a) blue ink
- b) Giemsa
- c) Lugol's solution
- d) Hematoxylin and eosin

3. What total magnification is obtained when using a 40× objective and a 10× eyepiece?

- a) 400×
- b) 10×
- c) 40×
- d) 4×

4. Electron microscopes use as their source of illumination:

- a) White light
- b) White or ultraviolet light
- c) Cathode radiation
- d) Electron beam

5. Electron microscopes:

- a) Use photons as their radiation source
- b) Do not require special specimen preparation
- c) Require specialized specimen preparation
- d) Use an electron beam as the radiation source

6. The electron microscope:

- a) It is a type of light microscope that allows observation of cells *in vitro* and *in vivo*
- b) Enables the study of cell and tissue topology as well as internal structures
- c) Allows observation of the same specimen in both SEM and TEM
- d) Has higher resolution than the confocal microscope; specimens must be specially prepared

7. Staining methods include:

- a) General/orientational
- b) Specific
- c) Random
- d) Universal

8. Phase contrast microscopy:

- a) Serves as the light source for low-contrast specimens
- b) Is used to enhance the contrast of certain cellular structures
- c) Is not applied in microscopy at all
- d) Enables observation of transparent objects with little intrinsic contrast

9. The inverted microscope:

- a) Is used, for example, in the observation of cell cultures
- b) Allows observation of specimens in transmitted light
- c) Is a type of electron microscope
- d) Uses illumination from above

10. Which component belongs to the mechanical part of the light microscope?

- a) Iris diaphragm
- b) Revolving nosepiece
- c) Condenser
- d) Objective

11. Which components belong to the illumination system of the microscope?

- a) Body tube
- b) Objectives
- c) Light source
- d) Condenser

12. The confocal microscope:

- a) Is a form of light fluorescence microscope with improved resolution
- b) Uses lasers with different excitation wavelengths as the light source
- c) Allows selective focusing and imaging of a specific optical plane in thicker sections
- d) Is a type of electron microscope

13. Which of the following methods is most suitable for obtaining a surface image of a specimen?

- a) SEM – Scanning electron microscope
- b) TEM – Transmission electron microscope
- c) Inverted microscope
- d) Light microscope

14. Which method is used for visualizing living cells?

- a) SEM – Scanning electron microscope
- b) TEM – Transmission electron microscope
- c) Phase contrast microscope
- d) All of the above

15. Which parts of the microscope provide magnification?

- a) Objective
- b) Iris diaphragm
- c) Condenser
- d) Eyepiece

16. Which microscope has the illumination and optical systems arranged in reverse order?

- a) electron microscope
- b) scanning microscope
- c) inverted microscope
- d) raster microscope

17. The optical parts of the microscope include:

- a) eyepiece
- b) revolving nosepiece
- c) light source
- d) objective

18. The mechanical parts of the microscope include:

- a) body tube
- b) revolving nosepiece
- c) fine adjustment knob
- d) coarse adjustment knob

19. The mechanical parts of the microscope include:

- a) coarse adjustment knob
- b) movable stage
- c) eyepieces
- d) objectives

20. Orientation in the field of view is based on:

- a) division into concentric circles
- b) division into quadrants
- c) orientation by the dial of a clock
- d) iris diaphragm

21. Native preparations:

- a) are used for observing physiological manifestations of cells
- b) are also used for observing the typical morphology of cells
- c) allow detailed observation of intracellular structures
- d) allow observation of cell motility

22. The image in the microscope is:

- a) inverted
- b) magnified
- c) reduced
- d) real

23. The optical components of the electron microscope:

- a) are made of glass
- b) consist of ground glass lenses
- c) consist of electromagnetic lenses
- d) are impregnated with electron-dense agents

24. The optical components of the light microscope:

- a) are made of glass
- b) consist of ground glass lenses
- c) consist of electromagnetic lenses
- d) are impregnated with electron-dense agents

25. The illumination system of the light microscope includes:

- a) eyepiece and objective
- b) light source
- c) system of filters and diaphragms
- d) condenser

26. According to the preparation method, specimens may be:

- a) impression preparations
- b) smears
- c) covered with a coverslip
- d) permanent preparations

27. According to construction type, light microscopes are classified as:

- a) simple magnifiers and stereomicroscopes
- b) microscopes with one or more objectives
- c) electron microscopes
- d) inverted microscopes

28. According to the type of radiation used, microscopes are classified as:

- a) light microscopes
- b) electron microscopes
- c) cathode-ray microscopes
- d) discharge microscopes

29. The working distance is:

- a) the distance of the eye from the microscope
- b) the distance of the specimen from the front lens of the objective
- c) the distance between the objective and the eyepiece
- d) decreases with increasing objective magnification

30. To enhance structures in native preparations, one may use:

- a) dark-field microscopy
- b) phase contrast
- c) vital staining
- d) electron microscopy

31. To enhance structures in native preparations, one may use:

- a) bright-field microscopy
- b) phase contrast staining
- c) vital staining or phase contrast
- d) electron microscopy

32. In dark-field microscopy:

- a) a special type of condenser is used
- b) the condenser of the microscope transmits only lateral light
- c) only rays reflected from the object are observed
- d) phase contrast allows observation of fluorescent objects

33. Light microscopes use as a source of radiation:

- a) white light
- b) ultraviolet radiation
- c) cathode radiation
- d) electron beam

34. The light microscope consists of:

- a) optical system
- b) illumination system
- c) mechanical system
- d) biological system

35. Light microscopes are commonly used to observe objects:

- a) smaller than 70 μm
- b) larger than 70 μm
- c) smaller than 70 nm
- d) not visible to the naked eye

36. The transmission electron microscope:

- a) has a ground glass lens, uses an electron beam, and scans the specimen surface
- b) has a ground glass lens, uses an electron beam, and can also observe the interior of a cell
- c) requires preparation of the specimen with an electron-dense substance that emits excess energy after UV absorption
- d) contains an electromagnetic lens and enables observation of the internal structures of the cell

37. The final magnification in microscopy is calculated as:

- a) the sum of the objective magnification and the working distance
- b) the product of the lens magnification and the objective magnification
- c) the product of the objective magnification and the eyepiece magnification
- d) the product of the working distance of the objective and the thickness of the slide

38. What is the main purpose of fixation of microscopic preparations?

- a) to increase the contrast of the specimen during observation
- b) to preserve the structure of cells and tissues against damage or decomposition
- c) to improve transmission of light through the specimen
- d) to reduce specimen thickness to an optimal level

39. What does the term resolution of a microscope express?

- a) the maximum magnification attainable
- b) the minimum distance between two points that can still be seen as separate
- c) the ability of the microscope to capture as much light as possible
- d) the ability to increase contrast without staining the specimen

40. What applies to the numerical aperture of an objective?

- a) a higher numerical aperture means less light entering the objective
- b) numerical aperture does not depend on the medium used between the specimen and the objective
- c) higher numerical aperture means better contrast and resolution
- d) the numerical aperture expresses only the theoretical maximum magnification of the objective

41. What causes spherical aberration?

- a) irregular refraction of light at the boundary of different media
- b) differential refraction of light through the center and periphery of the lens
- c) appearance of colored fringes at the edges of objects
- d) incorrect placement of the specimen on the slide

42. How does chromatic aberration manifest?

- a) blurred image only in one direction
- b) reduced specimen contrast
- c) colored fringes at the edges of observed objects
- d) blurred image at high magnification, even when using immersion oil

43. What kind of image is produced by lens astigmatism?

- a) sharp in one direction and blurred in the other
- b) with colored fringes at the edges
- c) blurred uniformly across the entire field
- d) high-contrast image but with low magnification

44. Confocal microscopy compared with conventional fluorescence microscopy:

- a) it uses polarized light to highlight structures
- b) it employs a pinhole aperture to eliminate scattered light and obtain optical sections
- c) it achieves resolution down to 0.2 nm using an electron beam
- d) it does not require fluorescent dyes for specimen imaging

45. Why is magnification alone insufficient without adequate resolution?

- a) because the image remains large but without detail
- b) because magnification always reduces contrast
- c) because high magnification is possible only in electron microscopy
- d) because magnification cannot be combined with staining of specimens

46. What is the main function of microscope calibration using an ocular micrometer?

- a) to increase specimen contrast
- b) to determine the dimensions of microstructures precisely
- c) to extend the lifespan of the microscope
- d) to distinguish between native and fixed specimens

47. Which substance is most often used to fix specimens in order to preserve cell and tissue structure?

- a) glycerol
- b) formalin
- c) agar
- d) methylene blue

48. What is the function of the immersion medium in microscopy?

- a) increases the lifespan of optical parts
- b) improves resolution and contrast at high magnifications
- c) enables observation of living cells
- d) reduces the need for microscope calibration

49. What risk does spherical aberration pose in microscopy?

- a) appearance of colored fringes at the image edges
- b) disintegration of specimen structure
- c) sharp image only in one direction
- d) blurred image because light rays do not refract evenly

50. What characterizes the numerical aperture (NA) of an objective?

- a) its ability to gather light depending on the angle of rays and the refractive index of the medium
- b) the maximum number of observable cells
- c) the speed of refocusing when working with the objective
- d) the size of the specimen suitable for observation

51. What problem is caused by chromatic aberration?

- a) the image is blurred
- b) the image shows colored fringes at the edges
- c) the image is sharp only in one direction
- d) the image appears three-dimensional

52. How should microscope objectives and eyepieces be cleaned?

- a) with ordinary tissue paper
- b) with special wipes or solutions designed for optics
- c) with industrial-grade alcohol
- d) with distilled water

53. What is the primary function of the microscope objective?

- a) to magnify the image of the object
- b) to direct light rays onto the specimen
- c) to protect the eyepiece from damage
- d) to regulate light intensity

54. What is the function of the microscope stage?

- a) to ensure correct placement of the specimen
- b) to increase observation contrast
- c) to provide a mechanism for changing objectives
- d) to regulate electrical voltage

55. Which part of the microscope is used to change objectives?

- a) body tube
- b) eyepiece
- c) revolving nosepiece
- d) condenser

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. FTTF | 20. TTTF | 39. FTFF |
| 2. FTFF | 21. TTFT | 40. FFTF |
| 3. TFFF | 22. TTFF | 41. FTFF |
| 4. FFFT | 23. FFTF | 42. FFTF |
| 5. FFTT | 24. TTFF | 43. TFFF |
| 6. FTFT | 25. FTTF | 44. FTFF |
| 7. TTFF | 26. TTTT | 45. TFFF |
| 8. FTFT | 27. TTFT | 46. FTFF |
| 9. TTFT | 28. TTFF | 47. FTFF |
| 10. FTFF | 29. FTFT | 48. FTFF |
| 11. FFTT | 30. TTTF | 49. FFFT |
| 12. TTTF | 31. FFTF | 50. TFFF |
| 13. TFFF | 32. TTTF | 51. FTFF |
| 14. FFTF | 33. TTFF | 52. FTFF |
| 15. TFFT | 34. TTTF | 53. TFFF |
| 16. FFTF | 35. TFFT | 54. TFFF |
| 17. TFFT | 36. FFFT | 55. FFFT |
| 18. TTTT | 37. FFTF | |
| 19. TTFF | 38. FTFF | |

2 Structure and functional organization of prokaryotic and eukaryotic cells. Molecules in cells and organisms

The **cell** (Latin *cellula*, Greek *kytos*) is the fundamental structural, functional, and reproductive unit of all living organisms, whether unicellular or multicellular. It is an autonomous and dynamic system that exhibits the fundamental manifestations of life, including metabolism, growth, responsiveness, reproduction, and development. The scientific discipline focused on the study of cells is called cytology.

The term cell was first used by Robert Hooke in 1665, when he observed the structure of cork using a simple microscope. During the 18th and 19th centuries, cytology developed into an independent field. In 1838, Matthias Schleiden and Theodor Schwann formulated the cell theory, which established that the cell is the basic unit of all living organisms. In 1855, Rudolf Virchow extended this theory with the principle that every cell arises from a pre-existing cell – “*Omnis cellula e cellula*.”

Advances in microscopy, particularly the development of electron microscopy, made it possible to gain a more precise understanding of cell structure. In general, all cells consist of a genetic apparatus (nuclear region), cytoplasm, and a cytoplasmic membrane. Based on the organization of the nucleus and other structures, cells are classified as prokaryotic or eukaryotic.

Prokaryotic cells, which form unicellular organisms such as bacteria and cyanobacteria, have a simple structure. They lack a true nucleus but contain a nucleoid – a region with a single circular DNA molecule. They also contain extrachromosomal DNA in the form of plasmids. Prokaryotes lack membrane-bound organelles but possess ribosomes with a smaller sedimentation coefficient (70S). Their cell wall is composed of peptidoglycan, which plays a key role in recognition by the host immune system. Additional surface structures may be present, including pili, fimbriae, flagella, and a capsule. Prokaryotic cells reproduce primarily asexually, most commonly by binary fission following DNA replication. Some bacteria also undergo **parasexual processes** (horizontal gene transfer) such as conjugation, during which DNA is exchanged between cells, significantly contributing to the spread of antibiotic resistance genes. Prokaryotic cells occur in a variety of shapes, including spherical cocci and rod-shaped bacilli. Bacteria are further classified according to Gram staining as Gram-positive or Gram-negative, an important criterion for their identification.

Eukaryotic cells, which constitute both unicellular and multicellular organisms, have a more complex structure. They contain a true nucleus, separated from the cytoplasm by a nuclear envelope, and a variety of membrane-bound organelles, including mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, lysosomes, and peroxisomes. An important component is the cytoskeleton, which forms the dynamic framework of the cell. Eukaryotic cells are the fundamental building blocks of fungi, plants, and animals; their bodies are organized into tissues that form organs and organ systems. Eukaryotic cells exhibit diverse shapes and sizes,

which are genetically determined and dependent on their function within the organism. The basic form is spherical, from which other shapes are derived, such as disc-like, flat, cuboidal, cylindrical, polygonal, and many more. Their size may be small, medium, or large – for example, human erythrocytes are small, while the oocyte is large. Cells may also possess projections characteristic of their specialized roles.

The following table summarizes the fundamental differences between prokaryotic and eukaryotic cells.

	Prokaryotic Cell	Eukaryotic Cell
Nucleus	Nucleoid (free DNA in the cytoplasm, no nuclear membrane)	True nucleus, DNA enclosed by a double nuclear membrane
DNA	Circular, often with plasmids	Linear, organized into chromosomes
Organelles	No membrane-bound organelles (only 70S ribosomes)	Membrane-bound organelles (mitochondria, ER, Golgi apparatus, lysosomes, chloroplasts in plants), 80S ribosomes
Size	Smaller (1–10 μm)	Larger (10–100 μm)
Reproduction	Asexual (binary fission); parasexual mechanisms (conjugation, transformation, transduction)	Sexual and asexual (mitosis, meiosis)
Surface structures	Pili, fimbriae, flagella, capsule	Microvilli, cilia, flagella (structurally different from prokaryotic flagella)
Cell wall	Usually present (peptidoglycan; Gram+ or Gram–)	Cellulose in plants and chitin in fungi; absent in animals
Examples	Bacteria, cyanobacteria	Protists, fungi, plants, animals

Molecules in cells and organisms constitute the basic building blocks of life and are essential for all biological processes. These include biopolymers such as proteins, nucleic acids, and polysaccharides, as well as lipids, small organic molecules known as metabolites, and inorganic molecules and ions. In biology, molecules are categorized by size as small molecules or large molecules (macromolecules).

Small molecules are characterized by their relatively low molecular weight, simple structure, and rapid diffusion, whereas **macromolecules** have higher molecular weights, polymeric structures, and more complex functions. Among the most notable small molecules are water, the principal constituent of cells (60–80%), and ATP, the universal molecule of energy metabolism. Cyclic adenosine monophosphate (cAMP) is an important second messenger in cell signaling. It is generated from ATP by the enzyme adenylate cyclase and activates protein kinase A (PKA), thereby regulating metabolism, gene expression, and cellular responses to hormones and neurotransmitters.

The following table provides an overview of major molecular groups.

Group	Examples	Functions
Small molecules	Water, ions (Na ⁺ , K ⁺ , Ca ²⁺), ATP, GTP, NADH, glucose, fatty acids	Metabolism, energy transfer, signaling, reaction environment
Large molecules	Proteins, DNA, RNA, polysaccharides, and membrane lipids	Enzymatic catalysis, genetic information, structural roles, and regulation

Proteins are macromolecules composed of amino acids linked by peptide bonds. They play essential roles in all aspects of cellular life, from catalyzing chemical reactions as enzymes to forming structural components that provide cells and tissues with shape and mechanical strength. Proteins also function as transport molecules, signaling mediators, and defense mechanisms against pathogens.

Nucleic acids, which include DNA and RNA, are carriers of genetic information. DNA stores the long-term genetic instructions necessary for the growth, development, and reproduction of organisms. At the same time, RNA transfers this information from DNA and participates in the process of protein synthesis. DNA contains the instructions for the synthesis of all proteins required by the organism, whereas RNA ensures their expression within the cell.

Polysaccharides are complex carbohydrates composed of many monosaccharide units and serve as storage substances or structural components. In plants, polysaccharides such as starch store energy, while cellulose provides rigidity to the cell wall. In animal cells, glycogen fulfills a similar role as a rapidly mobilizable energy reserve.

Lipids are a diverse group of molecules that are insoluble in water but soluble in organic solvents. They include phospholipids and sterols. Phospholipids form the structural basis of cell membranes, which separate the intracellular environment from the external milieu and regulate the passage of substances across the membrane. Fats and oils serve as important energy storage molecules, whereas sterols, such as cholesterol, are essential for maintaining membrane stability and regulating hormones.

Metabolites are small organic molecules produced during cellular metabolic processes. They serve as precursors for more complex structures or as energy sources for the cell. Primary metabolites are indispensable for basic cellular functions, while secondary metabolites often fulfill specialized roles, such as defense against pathogens or intercellular communication.

Inorganic molecules and ions, such as water and electrolytes (for example, sodium, potassium, and calcium), are also integral to the cellular environment. Water constitutes the majority of a cell's mass and serves as the medium in which all biochemical reactions occur. Ions play a

crucial role in maintaining the balance between the intracellular and extracellular environment, in signal transmission, and in numerous enzymatic processes.

The following table summarizes the principal classes of biomolecules in the cell.

Group	Composition	Functions	Examples
Proteins	Amino acids linked by peptide bonds	Enzymes, structural support, transport, signaling, immunity	Hemoglobin, collagen, insulin
Nucleic acids	Nucleotides (sugar + phosphate + base)	Transmission of genetic information	DNA, mRNA, tRNA, rRNA
Polysaccharides	Monosaccharides linked by glycosidic bonds	Energy storage, structural support	Starch, glycogen, cellulose, chitin
Lipids	Fatty acids, glycerol, sterols	Membranes, energy storage, hormones	Phospholipids, cholesterol, and triglycerides
Metabolites	Small organic molecules	Primary – basic metabolism; secondary – specialized functions	ATP, amino acids, vitamins, alkaloids
Inorganic substances	Water, mineral ions	Medium for reactions, homeostasis, signaling	H ₂ O, Na ⁺ , K ⁺ , Ca ²⁺ , Mg ²⁺ , Cl ⁻



Model questions

1. Which organisms are composed of eukaryotic cells?

- a) plants
- b) fungi
- c) animals
- d) multicellular organisms

2. What is the name of the metabolic process in the cell in which new protein molecules are synthesized?

- a) protein synthesis
- b) nucleolysis
- c) photosynthesis
- d) oxidative phosphorylation

3. Cellular compartmentalization is characteristic of which cells?

- a) prokaryotic
- b) eukaryotic
- c) bacterial
- d) human epithelial cells

4. Which statements about proteins are correct?

- a) proteins are composed of amino acids linked by peptide bonds
- b) proteins can function as enzymes
- c) proteins are the primary carriers of genetic information
- d) proteins have a lower molecular weight compared with lipids

5. Which statement about lipids is correct?

- a) lipids are soluble in water and insoluble in organic solvents
- b) phospholipids form the basis of cell membranes
- c) fats and oils serve as energy storage molecules
- d) sterols contribute to membrane stability and hormonal regulation

6. Which statements apply to prokaryotic cells?

- a) they have a nucleus enclosed by a membrane
- b) they contain mitochondria and chloroplasts
- c) DNA is present as a single circular molecule
- d) they contain 70S ribosomes

7. Which structures or properties are characteristic of eukaryotic cells?

- a) nucleus enclosed by two double membranes
- b) presence of cytoskeleton
- c) exclusively asexual reproduction by binary fission
- d) ribosomes with a sedimentation constant of 80S

8. What are the differences between prokaryotic and eukaryotic cells?

- a) prokaryotes have a cell wall made of peptidoglycan, eukaryotes (if they have one) of cellulose or chitin
- b) prokaryotes lack membrane-bound organelles, eukaryotes possess several types
- c) prokaryotes have smaller ribosomes than eukaryotes
- d) eukaryotes store genetic information in multiple circular DNA molecules

9. Which polysaccharide serves as the storage substance in animal cells?

- a) cellulose
- b) glycogen
- c) starch
- d) chitin

10. Small cells reach a size of:

- a) up to 10 μm
- b) 30 μm
- c) more than 30 μm
- d) 10 to 30 μm

11. Which of the following applies to the type and number of proteins on and in the cytoplasmic membrane?

- a) the number and type of proteins don't change
- b) the number and type of proteins changes
- c) the cell actively regulates them
- d) the cell passively acquires them

12. Prokaryotic cells:

- a) form the bodies of multicellular organisms
- b) are simpler than eukaryotic cells
- c) have their genome in a single circular DNA molecule
- d) lack ribosomes

13. Large cells reach a size of:

- a) 10 μm
- b) 5 μm
- c) more than 30 μm
- d) 10 to 30 μm

14. Prokaryotic ribosomes:

- a) are smaller than eukaryotic ribosomes
- b) are composed of two subunits
- c) are composed of three types of rRNA
- d) have a sedimentation constant of 80S

15. The prokaryotic cell consists of:

- a) cell wall
- b) cytoplasmic membrane
- c) nuclear material
- d) ribosomes

16. The prokaryotic chromosome is composed of:

- a) chromatin and non-histone proteins
- b) double-stranded DNA molecule
- c) DNA with histone and non-histone proteins
- d) single-stranded DNA molecule

17. In which type of cells are vacuoles found?

- a) in plant cells
- b) in eukaryotic cells
- c) in human somatic cells
- d) in animal cells

18. Are genes of eukaryotic cells also located outside the nucleus?

- a) yes
- b) no
- c) in mitochondria
- d) in chloroplasts

19. The basic shape of cells:

- a) is spherical
- b) has the largest volume with the smallest surface area
- c) has the largest surface area with the smallest volume
- d) is polygonal

20. What is the name of the science that studies the structure and functions of cells?

- a) anatomy
- b) physiology
- c) cytology
- d) molecular biology

21. Which organelles are found only in plant cells but not in animal cells?

- a) mitochondria
- b) chloroplasts
- c) endoplasmic reticulum
- d) ribosomes

22. Among specialized cell shapes are:

- a) truncated pyramids
- b) cuboidal
- c) flat
- d) stellate

23. Irregular cytoplasmic projections are characteristic of:

- a) erythrocytes
- b) pericytes
- c) leukocytes
- d) oocytes

24. The size of cells:

- a) is genetically determined for the given species
- b) is determined by cell differentiation
- c) is unrelated to the size of the organism
- d) is influenced by the size of the organism; larger organisms generally have larger cells

25. Every cell consists of:

- a) cell surface
- b) cytoplasm
- c) nucleolus
- d) cytoskeleton

26. What is the main role of adenosine triphosphate (ATP) in the cell?

- a) it serves as a storage form of genetic information
- b) it is the basic building block of proteins
- c) it represents the universal energy source for cellular processes
- d) it ensures oxygen transport in the blood

27. What is the main role of cyclic adenosine monophosphate (cAMP) in the cell?

- a) it serves as a storage form of genetic information
- b) it is an important second messenger in cell signaling
- c) it represents the universal energy source for cellular processes
- d) it activates protein kinase A

28. Which of the following functions belongs to lipids?

- a) direct transfer of genetic information
- b) formation of biological membranes
- c) catalysis of biochemical reactions as enzymes
- d) storage of genetic information

29. The basic functions of proteins include:

- a) structural
- b) transport
- c) metabolic
- d) protective

30. What constitutes the primary structure of nucleic acids?

- a) spatial arrangement of the DNA double helix
- b) sequence of nucleotides in a polynucleotide chain
- c) bonds between histones and DNA in chromatin
- d) three-dimensional arrangement of tRNA

31. What characterizes the secondary structure of proteins?

- a) linear sequence of amino acids in a polypeptide chain
- b) spatial arrangement of the chain into α -helices and β -sheets
- c) three-dimensional arrangement of the entire polypeptide
- d) association of multiple polypeptide chains into a functional complex

32. Which statement about amino acids is correct?

- a) they are the basic building blocks of nucleic acids
- b) they always contain a carboxyl group and an amino group
- c) they serve as precursors for the synthesis of hormones and neurotransmitters
- d) all amino acids are essential for the human body

33. What role do phospholipids play in the cell?

- a) they are the basis of genetic information
- b) they form the structural basis of cell membranes
- c) they serve as the main storage polysaccharide
- d) they transport oxygen in the blood

34. Water:

- a) has a high heat capacity
- b) is an effective medium for the transport of thermal energy
- c) has a transport function
- d) is an excellent solvent for many chemicals

35. Which cells have fibrous projections?

- a) motor neurons
- b) pyramidal cells
- c) sperm cells
- d) leukocytes

36. A cell can be defined as:

- a) a system with goal-directed behavior
- b) an autologous system
- c) an open system
- d) a system with self-reproduction

37. Medium-sized cells reach a size of:

- a) more than 30 μm
- b) 5 μm
- c) less than 10 μm
- d) 10 to 30 μm

38. What is the basic shape of the cell?

- a) flat
- b) cuboidal
- c) spherical
- d) cylindrical

39. Which of the following groups belong among the basic biomacromolecules?

- a) proteins, nucleic acids, polysaccharides, lipids
- b) proteins, vitamins, polysaccharides, inorganic substances
- c) nucleic acids, lipids, metabolites, mineral ions
- d) polysaccharides, amino acids, lipids, water

40. Which statement correctly describes the difference between prokaryotic and eukaryotic cells?

- a) prokaryotic cells have a true nucleus, eukaryotic cells do not
- b) prokaryotic cells contain membrane-bound organelles, eukaryotic cells do not
- c) prokaryotic cells have circular DNA, eukaryotic cells have linear chromosomes
- d) prokaryotic cells are always larger than eukaryotic cells

41. Which statements are correct?

- a) ATP is the universal energy currency of the cell
- b) cAMP is a second messenger in cell signaling
- c) ATP stores genetic information
- d) cAMP is the main energy reserve substance

42. Which substances are classified as macromolecules?

- a) proteins
- b) RNA
- c) potassium ions (K^+)
- d) water

43. What are the functions of proteins in the cell?

- a) catalyze biochemical reactions
- b) store genetic information
- c) ensure transport of substances
- d) act as the universal energy currency

44. Which of the following belong among the small molecules of the cell?

- a) water
- b) DNA
- c) sodium ions (Na^+)
- d) proteins

45. What is the role of ATP in the cell?

- a) cells store it in large amounts as an energy reserve
- b) it is the universal energy carrier
- c) it forms the basis of the cell wall in eukaryotes
- d) it mediates muscle contraction

46. Comparison of ribosomes:

- a) prokaryotic cells have 90S ribosomes
- b) eukaryotic cells have 80S ribosomes
- c) prokaryotic cells have 70S ribosomes
- d) eukaryotic cells lack ribosomes

47. Which surface structures may be present in prokaryotic cells?

- a) pili
- b) flagella
- c) nuclear membrane
- d) chloroplasts

48. What forms the cell wall of plant cells?

- a) chitin
- b) peptidoglycan
- c) cellulose
- d) proteoglycans

49. What is the main function of polysaccharides in plants?

- a) energy storage in the form of starch
- b) transmission of genetic information
- c) membrane formation
- d) catalysis of chemical reactions

50. Comparison of DNA:

- a) in prokaryotes, it is circular
- b) in eukaryotes it is linear
- c) in prokaryotes, it is linear
- d) in eukaryotes, it is always located in the cytoplasm

51. Which biomolecules are composed of amino acids linked by peptide bonds?

- a) lipids
- b) proteins
- c) polysaccharides
- d) nucleic acids

52. Which statement correctly describes the difference between prokaryotic and eukaryotic cells?

- a) prokaryotes have a nucleus; eukaryotes do not
- b) prokaryotes have a nucleoid; eukaryotes have a true nucleus
- c) prokaryotes have mitochondria, eukaryotes do not
- d) prokaryotes have chromosomes; eukaryotes have plasmids

53. What is the difference in genetic material between prokaryotes and eukaryotes?

- a) prokaryotes – linear DNA, eukaryotes – circular DNA
- b) prokaryotes – circular DNA, eukaryotes – linear DNA
- c) prokaryotes and eukaryotes always contain only one DNA molecule
- d) eukaryotes lack DNA

54. What applies to cell reproduction?

- a) prokaryotes – binary fission, eukaryotes – mitosis and meiosis
- b) prokaryotes – mitosis, eukaryotes – binary fission
- c) both prokaryotes and eukaryotes always reproduce sexually
- d) prokaryotes do not undergo any division

55. What is the difference in cell size?

- a) the size of cells is the same in both types
- b) prokaryotes are larger than eukaryotes
- c) eukaryotes are always smaller than viruses
- d) prokaryotes – 1–10 μm , eukaryotes – 10–100 μm

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. TTTT | 20. FFTF | 39. TFFF |
| 2. TFFF | 21. FTFF | 40. FFTF |
| 3. FTFT | 22. TFFT | 41. TTFF |
| 4. TTFF | 23. FTTF | 42. TTFF |
| 5. FTTF | 24. TTFT | 43. TFTF |
| 6. FFTT | 25. TTFF | 44. TFTF |
| 7. FTFT | 26. FFTF | 45. FTFT |
| 8. TTTF | 27. FTFT | 46. FTTF |
| 9. FTFF | 28. FTFF | 47. TTFF |
| 10. TFFF | 29. TTTT | 48. FFTF |
| 11. FTTF | 30. FTFF | 49. TFFF |
| 12. FTTF | 31. FTFF | 50. TTFF |
| 13. FFTF | 32. FTTF | 51. FTFF |
| 14. TTTF | 33. FTFF | 52. FTFF |
| 15. TTTT | 34. TTTT | 53. FTFF |
| 16. FTFF | 35. TTFF | 54. TFFF |
| 17. TTFF | 36. TFTT | 55. FFTT |
| 18. TFTT | 37. FFFT | |
| 19. TTFF | 38. FFTF | |

3 Biomembranes, substance transport, and intercellular communication. Membrane receptors

Cellular membranes (biomembranes) form the universal boundary of all cells and organelles. They are 6–10 nm thick, separate environments of differing composition, and ensure selective permeability, transport of substances, reception of signals, and mutual communication.

The fundamental structure of the membrane is the phospholipid bilayer, in which the hydrophilic heads face the aqueous environment and the hydrophobic fatty acid chains are oriented inward. This organized yet dynamic system enables membrane fusion (during endocytosis and exocytosis) and is sensitive to environmental changes (e.g., under hypotonic conditions). Membrane flexibility is enhanced by fluidity, which is regulated by cholesterol content.

Membrane proteins are divided into integral (embedded within or spanning the bilayer) and peripheral (bound electrostatically to the surface). They participate in transport, signal transduction, enzymatic reactions, and also form antigenic determinants. As required, they may cluster into functional assemblies known as **lipid rafts**.

Cell Junctions

Cells connect through specialized contacts: *zonula occludens* (tight junctions) and gap junctions, which function as barrier or communication junctions; *zonula adhaerens*, which encircles the cell and strengthens tissue cohesion; and *macula adhaerens* (desmosome), which forms disc-like, mechanically resilient attachments with tonofilaments. These structures ensure tissue integrity and coordination between cells.

The following table provides examples of cell junction types.

Type of Junction	Characterization	Function	Examples
Zonula occludens (tight junctions)	Close contact between the membranes of neighboring cells	Barrier, prevents the paracellular passage of substances	Intestinal epithelium, blood-brain barrier
Gap junctions	Channels formed by connexins linking cytoplasms	Direct exchange of ions and small molecules, electrical and metabolic communication	Myocardium, hepatocytes
Zonula adhaerens	Belt-like structure linked to actin filaments	Tissue reinforcement, cell-cell adhesion	Epithelial cells
Macula adhaerens (desmosome)	Disc-like structures with tonofilaments	Strongest adhesion, mechanical resistance	Epidermis, myocardium

Transport of Substances Across the Membrane

Transport may be passive or active. Passive transport proceeds without energy consumption along a gradient – diffusion and osmosis, or facilitated diffusion mediated by transport proteins or aquaporins. Active transport occurs against the gradient and requires ATP: primary active transport (Na^+/K^+ pump, Ca^{2+} pump) and secondary active transport (cotransport via symport or antiport). Ion channels play a specific role, activated by changes in voltage, ligand binding, or mechanical stimuli.

Macromolecules cross the membrane via endocytosis, the process by which substances are taken up into the cell. Pinocytosis involves the uptake of soluble molecules, whereas phagocytosis involves the uptake of solid particles. The release of substances into the extracellular environment is termed exocytosis.

Intercellular Communication

Cells communicate via chemical signals and their receptors. Signals must be highly specific to ensure the accuracy of the response. Neural communication involves the rapid release of neurotransmitters from a neuron to a target cell through a synapse. Humoral communication occurs via signaling molecules in the extracellular space.

Humoral communication can be classified as: **endocrine** – hormones transported by the bloodstream act on distant cells, **paracrine** – local action on neighboring cells within a tissue, **autocrine** – regulation of a cell's own activity by its own signals, and **direct intercellular signaling** – through gap junctions or plasmodesmata.

Membrane receptors

Membrane receptors are specialized protein structures embedded in the cytoplasmic membrane that mediate the reception of signals from the extracellular environment and their conversion into intracellular responses. They enable cells to respond to hormones, growth factors, cytokines, and neurotransmitters, thereby influencing vital processes. Membrane receptors regulate metabolism, growth, differentiation, and apoptosis; they mediate intercellular communication during tissue development and regeneration; and they play a pivotal role in immune processes through receptors for antigens and cytokines.

Each receptor consists of three main regions: an extracellular domain, which binds the ligand (the so-called *first messenger*); a transmembrane domain, which anchors the receptor within the lipid bilayer; and an intracellular domain, which transmits the cellular response. Upon ligand binding, the receptor protein undergoes conformational changes that initiate a signaling cascade. Several principal classes of membrane receptors are distinguished into the following types.

Ligand-gated ion channel receptors. The binding of a ligand opens the channel, allowing the passage of ions (Na^+ , K^+ , Cl^- , Ca^{2+}). Examples include the nicotinic acetylcholine receptor and receptors for glycine and GABA. Their typical function is to alter the permeability of ions across the membrane.

Enzyme-linked receptors. Ligand binding activates intrinsic enzymatic activity, most often phosphorylation. A typical example is receptors with tyrosine kinase activity (e.g., the insulin receptor and receptors for growth factors). Activation frequently leads to receptor dimerization and initiation of phosphorylation cascades.

G protein-coupled receptors (GPCRs). These represent the largest class of membrane receptors. They are characterized by seven transmembrane helices, an extracellular ligand-binding domain, and an intracellular region that interacts with a G protein. Upon activation, the G protein undergoes GDP/GTP exchange and subsequently modulates effector enzymes such as adenylate cyclase or phospholipase C. This results in the generation of second messengers (cAMP, IP_3 , DAG, Ca^{2+}).



Model questions

1. If peripheral proteins:

- a) carry a negative charge, they attach directly to the membrane
- b) carry a negative charge, they attach to the membrane via Ca^{2+} ions
- c) carry a positive charge, they attach directly to the membrane
- d) carry a positive charge, they attach to the membrane via Ca^{2+} ions

2. What is the main role of receptors in cells?

- a) to transmit signals from the external environment into the cell
- b) to regulate ATP levels in the cell
- c) to control cell motility
- d) to enable protein synthesis

3. Which substances/molecules are transported by vesicular transport?

- a) large particles – e.g., fragments of organelles
- b) macromolecules
- c) fluids
- d) all of the above

4. What are the properties of the cell membrane?

- a) protection
- b) semipermeability
- c) selective permeability
- d) separates the cell from its surroundings

5. What is the name of the phenomenon of red blood cell rupture?

- a) hemoptysis, occurs in a hypertonic environment
- b) hemolysis, occurs in an isoosmotic environment
- c) hemolysis, occurs in a hypotonic environment
- d) plasmolysis, occurs in a hypertonic environment

6. What is the environment called when the osmotic value of the medium is lower than that of the cell?

- a) hypertonic
- b) hypotonic
- c) isoosmotic
- d) isotonic

7. What is the environment called when the osmotic value of the medium is higher than that of the cell?

- a) hypertonic
- b) hyperosmotic
- c) isotonic
- d) hypotonic

8. What is the environment called when the osmotic value of the medium equals that of the cell?

- a) isotonic
- b) isoosmotic
- c) hypertonic
- d) hypotonic

9. What is the ability of a cell to engulf microorganisms called?

- a) diffusion
- b) phagocytosis
- c) osmosis
- d) pinocytosis

10. What is the general mechanism for releasing substances from cells?

- a) pinocytosis
- b) diffusion
- c) osmosis
- d) exocytosis

11. Active transport is:

- a) diffusion and osmosis
- b) movement of substances against their concentration gradient
- c) movement of substances requiring energy
- d) plasmolysis and cytolysis

12. A biological membrane is:

- a) composed of phospholipids and proteins
- b) the basis of membrane-bound organelles such as mitochondria and chloroplasts
- c) part of the cell wall
- d) the basis of membrane-bound organelles such as ribosomes

13. A biological membrane:

- a) is found only in eukaryotic cells
- b) is composed of a phospholipid bilayer and proteins
- c) in animal cells, it does not contain cholesterol
- d) performs various functions such as uptake of substances, cell-cell interactions, signal recognition, etc.

14. Biological membranes contain:

- a) phospholipids
- b) cholesterol
- c) peripheral and integral proteins
- d) polar lipids

15. Cell junctions include:

- a) macula occludens
- b) desmosome
- c) G proteins
- d) zonula adhaerens

16. Cell junctions are:

- a) cornea occludens
- b) desmatozome
- c) G proteins
- d) zonula adhaerens and desmosome

17. What are the proteins called that are embedded in the phospholipid bilayer or span across it?

- a) peripheral proteins
- b) integral proteins
- c) glycoproteins
- d) lipid rafts

18. What is osmotic lysis of a cell?

- a) the phenomenon when a cell takes up water, increases in volume, and bursts
- b) the phenomenon occurring when an animal cell is placed in a hypotonic environment
- c) the phenomenon when a cell shrinks upon water uptake
- d) the phenomenon when a cell does not change volume upon water uptake

19. Facilitated diffusion:

- a) is transport mediated by lipid droplets
- b) is the most common form of transport into the nucleolus
- c) occurs without energy expenditure along the concentration gradient
- d) consumes energy in the form of ATP

20. Facilitated diffusion:

- a) is transport mediated by plasma membrane proteins
- b) occurs without energy expenditure
- c) occurs along the concentration gradient
- d) occurs by binding of a substance to a transport protein at the cell surface, followed by conformational change and release into the cytoplasm

21. Types of chemical communication include:

- a) endocrine
- b) paracrine
- c) motocrine
- d) autocrine

22. Which processes belong to endocytosis?

- a) diffusion
- b) pinocytosis
- c) phagocytosis
- d) osmosis

23. Which substances can enter cells by free diffusion?

- a) gases
- b) urea
- c) ethanol
- d) oxygen

24. Which type of receptor mediates the effects of insulin?

- a) tyrosine kinase receptor
- b) nuclear receptor
- c) ion channel
- d) G protein-coupled receptor

25. Which mechanism is most typical for tyrosine kinase-associated receptors?

- a) change in ion permeability across the membrane
- b) receptor dimerization
- c) activation of cyclic AMP (cAMP)
- d) activation of cAMP kinase

26. Membrane receptors are classified into:

- a) receptors that are part of ion channels
- b) mechanoreceptors
- c) G protein-coupled receptors
- d) enzyme-linked receptors

27. For what purpose does the cell use osmotic energy?

- a) for transport of substances across membranes
- b) for the transport of substances during protein synthesis
- c) for the transport of substances during photosynthesis
- d) for transport of osmotically active substances

28. Which statements about the polarity of the biological membrane surface are correct?

- a) the biological membrane is nonpolar
- b) the biological membrane is polar
- c) the membrane surface carries a negative charge
- d) the membrane surface carries a positive charge

29. Osmosis is the process by which water passes through the cytoplasmic membrane from an environment:

- a) of lower concentration into one of higher concentration
- b) of higher concentration into one of lower concentration
- c) from a hypotonic to a hypertonic environment
- d) from a hypertonic to a hypotonic environment

30. The movement of molecules against a concentration gradient:

- a) requires energy, e.g., in the form of ATP
- b) is active transport
- c) continues until the concentrations on both sides of the membrane equalize
- d) is passive transport

31. G protein-coupled receptors:

- a) are not found in the plasma membrane of eukaryotic cells
- b) consist of polynucleotide chains spanning the membrane
- c) do not have an extracellular domain
- d) represent the most numerous group of membrane receptors

32. What is the role of cholesterol in the biological membrane?

- a) it is a major receptor
- b) it increases its flexibility
- c) it modulates fluidity and strengthens it
- d) it imparts a positive charge

33. What are biomembranes composed of?

- a) two layers of phospholipid molecules and protein molecules
- b) two layers of phospholipid molecules and RNA
- c) a phospholipid bilayer
- d) a single layer of phospholipids

34. What are the basic components of the plasma membrane?

- a) phospholipids, cholesterol, proteins, glycolipids
- b) calcium, cholesterol, cerebroside, gangliosides
- c) hormones, proteins, ATP, phospholipids
- d) peptidoglycans, chitin, cellulose, glycoproteins

35. Which part of a membrane receptor mediates the cellular response?

- a) extracellular domain
- b) intracellular domain
- c) transmembrane segment
- d) lipid bilayer

36. What is the typical function of ion channel-forming receptors?

- a) phosphorylation of proteins
- b) changing ion permeability across the membrane
- c) activation of G proteins
- d) initiation of apoptosis

37. Which statement about enzyme-linked receptors is correct?

- a) they contain seven transmembrane helices
- b) they most often catalyze phosphorylation
- c) they are activated by GTP binding
- d) they are typical receptors for the neurotransmitters GABA and glycine

38. Which of the following is an example of an ion channel receptor?

- a) insulin receptor
- b) nicotinic acetylcholine receptor
- c) β -adrenergic receptor
- d) epidermal growth factor receptor (EGFR)

39. What is the main role of second messengers in cell signaling?

- a) they transmit the signal from the extracellular space directly to the DNA
- b) they amplify and relay the signal between the receptor and intracellular effectors
- c) they serve as receptors for neurotransmitters
- d) they form the primary ligand-binding domain of membrane receptors

40. What is the significance of lipid rafts in cell signaling and immune processes?

- a) they serve as storage for cholesterol and fatty acids
- b) they ensure degradation of damaged proteins in the membrane
- c) they concentrate receptors and signaling molecules, enabling more efficient activation of pathways (e.g., during immune responses)
- d) they reduce membrane fluidity and thereby block signaling

41. Where are glycolipids located in the biomembrane, and what is their main function?

- a) cytoplasmic side; catalysis of biochemical reactions
- b) nuclear membrane; regulation of transcription
- c) extracellular side; cell recognition and antigenic determinants
- d) mitochondria; electron transport

42. What applies to the type and number of membrane proteins?

- a) they are always the same in all cells
- b) they change only during apoptosis
- c) they are variable and depend on differentiation and the cell cycle phase
- d) they depend only on the amount of cholesterol in the membrane

43. What is the significance of communication through intercellular junctions (gap junctions or plasmodesmata)?

- a) transmission of hormones over long distances
- b) activation of gene expression in the nucleus
- c) direct transfer of ions and small molecules between neighboring cells
- d) prevention of any exchange between cells

44. What is the principal mechanism by which receptors mediate a cellular response?

- a) oxidation of fatty acids
- b) activation or inactivation of proteins frequently via phosphorylation
- c) synthesis of DNA in the nucleus
- d) passive diffusion of water

45. Which enzyme catalyzes the transfer of a phosphate group from ATP to a protein?

- a) phosphatase
- b) protein kinase
- c) DNA polymerase
- d) telomerase

46. Which cell junctions provide the strongest mechanical anchoring between cells?

- a) gap junctions
- b) desmosomes (macula adhaerens)
- c) zonula occludens
- d) zonula adhaerens

47. Which types of signaling are classified as humoral (chemical) communication?

- a) endocrine
- b) paracrine
- c) synaptic
- d) autocrine

48. Which receptor type has 7 transmembrane helices and uses second messengers?

- a) enzyme-linked receptors
- b) ion channel receptors
- c) G protein-coupled receptors
- d) antigen receptors

49. What is a typical function of enzyme-linked receptors?

- a) catalyzing phosphorylation reactions
- b) opening ion channels
- c) binding antibodies
- d) transporting glucose

50. Which example belongs to ion channel receptors?

- a) nicotinic acetylcholine receptor
- b) insulin receptor
- c) EGF receptor
- d) G protein-coupled receptor

51. Which cell junction allows direct transfer of small molecules between neighboring cells?

- a) desmosomes
- b) zonula occludens
- c) gap junctions
- d) zonula adhaerens

52. Which three parts does a typical membrane receptor contain?

- a) extracellular domain, transmembrane segment, intracellular domain
- b) ribosomal subunit, cytoskeleton, phospholipid
- c) nuclear DNA, nucleosome, histone
- d) cytoplasm, nucleus, mitochondrion

53. What happens after a ligand binds to its receptor?

- a) the receptor is degraded immediately
- b) a conformational change occurs and a signaling cascade is initiated
- c) DNA replicates immediately
- d) the receptor is transported into the nucleus

54. What is the function of the extracellular domain of a receptor?

- a) to bind the ligand
- b) to mediate phosphorylation
- c) to anchor the receptor in the lipid bilayer
- d) to transmit the signal into the nucleus

55. What is the function of the intracellular domain of a receptor?

- a) to bind the ligand
- b) to anchor the receptor in the membrane
- c) to mediate the cellular response
- d) to produce ATP

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. FTTF | 20. TTTT | 39. FTFF |
| 2. TFFF | 21. TTFT | 40. FFTF |
| 3. TTTT | 22. FTTF | 41. FFTF |
| 4. TTTT | 23. TTTT | 42. FFTF |
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| 6. FTFF | 25. FTFF | 44. FTFF |
| 7. TTFF | 26. TFFT | 45. FTFF |
| 8. TTFF | 27. TFFT | 46. FTFF |
| 9. FTFF | 28. FTTF | 47. TTFT |
| 10. FFFT | 29. TFTF | 48. FFTF |
| 11. FTTF | 30. TTFF | 49. TFFF |
| 12. TTFF | 31. FFFT | 50. TFFF |
| 13. FTFT | 32. FFTF | 51. FFTF |
| 14. TTTT | 33. TFTF | 52. TFFF |
| 15. FTFT | 34. TFFF | 53. FTFF |
| 16. FFFT | 35. FTFF | 54. TFFF |
| 17. FTFF | 36. FTFF | 55. FFTF |
| 18. TTFF | 37. FTFF | |
| 19. FFTF | 38. FTFF | |

4 Organelles of the eukaryotic cell

The eukaryotic **cell** is the fundamental structural and functional unit of all multicellular animals, plants, and fungi, as well as some unicellular organisms. In contrast to prokaryotes, it is characterized by the presence of a nucleus enclosed by a double membrane and a system of internal membrane-bound organelles, which enable spatial and functional compartmentalization. This organization allows eukaryotic cells to differentiate and form complex tissues and organs. The following table summarizes the major organelles and their main functions.

Organelle	Main functions
Cytoplasm	The internal environment of the cell; it serves as a medium for metabolic reactions and the transport of substances.
Nucleus	The control center of the cell and storage of genetic information (DNA).
Nucleolus	Synthesis of rRNA, assembly of ribosomal subunits.
Mitochondria	ATP production (cellular energy metabolism), Krebs cycle, apoptosis, Ca^{2+} regulation.
Chloroplasts	Site of photosynthesis: synthesis of glucose and other organic compounds in plants.
Rough endoplasmic reticulum	Protein synthesis, initial modifications, and transport to the Golgi apparatus.
Smooth endoplasmic reticulum	Lipid and steroid synthesis, detoxification, carbohydrate metabolism, and Ca^{2+} storage.
Golgi apparatus	Post-synthetic modification of proteins and lipids, sorting and packaging into vesicles, and secretion.
Lysosomes	Intracellular digestion, degradation of damaged organelles, and foreign particles.
Peroxisomes	Detoxification, breakdown of fatty acids, metabolism of hydrogen peroxide.
Vacuoles (plants)	Maintenance of turgor, storage of substances, and degradation of metabolites.
Ribosomes	Protein synthesis according to mRNA.
Cytoskeleton	Maintenance of cell shape, motility, organelle and vesicle transport, and participation in cell division.
Centrosome	Organization of microtubules, formation of the mitotic spindle.
Cilia and flagella	Cell motility or movement of particles across the epithelial surface.

The **cytoplasm** is the semi-liquid internal environment of the cell, filling the space between the nucleus and the plasma membrane. It is composed predominantly of water (70–80%) and contains ions, organic molecules, enzymes, and proteins that create a dynamic medium for metabolic processes. The cytoplasm is not homogeneous – it alternates between a more fluid (sol) and a more viscous (gel) state, enabling flexibility during organelle movement and cell division. It serves both as the matrix that holds cellular organelles and as the site where most metabolic reactions occur.

The **nucleus** is the control center of the cell. It contains genetic information in the form of DNA organized into chromosomes. The nucleus is surrounded by the nuclear envelope, a double membrane. At certain points, the membranes fuse, forming nuclear pores that ensure the selective transport of RNA and proteins between the nucleus and cytoplasm. Inside the nucleus, DNA exists as **chromatin** – euchromatin (decondensed, transcriptionally active) and heterochromatin (condensed, transcriptionally inactive). This spatial organization regulates gene activity.

A key nuclear substructure is the **nucleolus**, the site of rRNA synthesis and assembly of ribosomal subunits. Once formed, these subunits are transported through nuclear pores into the cytoplasm, where they join to create functional ribosomes.

Ribosomes are universal protein-synthesizing organelles. Eukaryotic ribosomes have a sedimentation coefficient of 80S and are composed of two subunits, a large and a small subunit, each consisting of rRNA and proteins. They may be free in the cytoplasm – synthesizing proteins for the cell's internal needs – or bound to the rough endoplasmic reticulum, producing proteins destined for secretion or membrane integration. Prokaryotic ribosomes contain three types of rRNA, whereas eukaryotic ribosomes contain four. The eukaryotic ribosome is composed of a 40S small subunit (with 18S rRNA and ~30 proteins) and a 60S large subunit (containing 28S rRNA, 5.8S rRNA, 5S rRNA, and ~45 proteins). The designation “S” refers to the Svedberg sedimentation coefficient, reflecting particle size and density.

The **endoplasmic reticulum (ER)** is a membrane network of cisternae and tubules that is continuous with the nuclear envelope. Two types are distinguished. The **rough ER** bears ribosomes on its surface and serves as the site of protein synthesis for secretion or membrane insertion. Newly synthesized proteins enter the ER lumen, where their initial modifications take place. The **smooth ER** lacks ribosomes and is responsible for the synthesis of lipids, steroids, and phospholipids, as well as the detoxification of xenobiotics. In muscle cells, a specialized form of the sarcoplasmic reticulum regulates Ca^{2+} storage and plays a crucial role in muscle contraction.

The **Golgi apparatus** consists of stacks of flattened cisternae organized into functional units called dictyosomes. It receives products from the ER, modifies them (e.g., glycosylation, phosphorylation), sorts and packages them into transport vesicles. Proteins and lipids arrive at the cis face, undergo modification in the medial cisternae, and are exported from the trans face to their destination – either the plasma membrane, lysosomes, or other organelles. The Golgi apparatus thus functions as the central hub of the cellular secretory system.

Lysosomes are vesicles containing hydrolytic enzymes capable of degrading proteins, polysaccharides, lipids, and nucleic acids. They ensure intracellular digestion, the breakdown of damaged organelles (**autophagy**), and the degradation of foreign particles taken up by phagocytosis. Defects in lysosomal enzymes cause the accumulation of indigestible material and lead to so-called **storage diseases**.

Peroxisomes are small membrane-bound organelles that contain oxidative enzymes. They catalyze the breakdown of fatty acids, participate in detoxification, and regulate the metabolism of hydrogen peroxide.

In plant cells, **vacuoles** have a specific role. Their content, known as **cell sap**, consists of ions, metabolites, and pigments. Vacuoles maintain turgor, store substances, and mediate recycling. In animal cells, vacuoles appear only as small and transient vesicles, serving primarily digestive and transport functions.

Mitochondria are the energy centers of the cell. Their number and shape (ovoid or filamentous) vary according to the cell's energy demand; for example, muscle cells contain thousands. They are enclosed by two membranes: a smooth outer membrane and a highly folded inner membrane forming **cristae**. The inner membrane houses enzymes of the respiratory chain and ATP synthase, which produces ATP. Mitochondria contain their own circular DNA and prokaryotic-like ribosomes, enabling partial autonomy in protein synthesis. This supports the **endosymbiotic theory**, which posits that mitochondria originated from the engulfment of free-living prokaryotes. Beyond energy metabolism, mitochondria are involved in apoptosis (programmed cell death) and calcium homeostasis.

Chloroplasts occur only in plant cells and are responsible for photosynthesis. They contain the green pigment chlorophyll, which converts light energy into chemical energy stored in glucose. A double membrane encloses chloroplasts, and their interior consists of the **stroma** and the thylakoid system organized into **grana**. Like mitochondria, they possess their own DNA and ribosomes, further supporting their endosymbiotic origin. In addition to photosynthesis, chloroplasts also contribute to the synthesis of certain amino acids, lipids, and pigments.

The **cytoskeleton** is a dynamic protein network that provides intracellular organization, facilitates organelle transport, and maintains cell shape. It is composed of **microtubules, microfilaments, and intermediate filaments**, each with specific functions that complement one another. **Microtubules** are hollow tubes built from tubulin heterodimers. They are crucial for cell division (formation of the mitotic spindle) and serve as “tracks” for intracellular transport. Organelles and vesicles move along microtubules through the action of motor proteins, such as dynein and kinesin. **Microfilaments**, composed of actin, are the thinnest cytoskeletal filaments. They drive cell motility, contraction, endocytosis, and exocytosis. Frequently forming a dense network beneath the membrane, they generate protrusions such as lamellipodia and filopodia, which are important for cell migration. **Intermediate filaments** provide mechanical strength and resistance to stress. Unlike microtubules and microfilaments, they are less dynamic and form a stable scaffold. Their composition varies by cell type – for example, keratins in epithelial cells or vimentin in mesenchymal cells.

The cytoskeleton is constantly remodeled in response to cellular needs and external signals. Besides its structural roles, it participates in signaling, regulates the cell cycle, and mediates interactions between cells and the extracellular environment.

Some eukaryotic cells possess motile organelles, such as **cilia and flagella**, which enable cell movement or the transport of particles across epithelial surfaces. Their structural basis is the **axoneme**, characterized by a “9 + 2” arrangement of nine peripheral microtubule doublets surrounding two central microtubules. Stability is ensured by proteins such as nexins, while dynein motor proteins convert ATP into the mechanical force required for bending movements.

Cilia are short and numerous, producing rhythmic beating. They move mucus and trapped particles in the respiratory tract and assist in egg transport in the oviduct. They may also act as sensory organelles. **Flagella** are longer and typically occur singly or in small numbers. A classic example is the sperm flagellum, essential for motility and fertilization. Defects in the structure and function of cilia or flagella cause disorders known as **ciliopathies**, which can affect the respiratory, reproductive, and nervous systems.



Model questions

1. Which organelles are typical for all eukaryotic cells?

- a) cytoskeleton composed of actin, tubulin, and intermediate filaments
- b) a true nucleus that is not enclosed by a membrane
- c) endomembrane system
- d) glycocalyx

2. Which organelles and structures are present in both prokaryotic and eukaryotic cells?

- a) mitochondria, true nucleus, ribosomes, cytoplasm
- b) ribosomes, cytoplasm, plasma membrane, glycocalyx
- c) ribosomes, cytoplasm, plasma membrane, DNA
- d) true nucleus, ribosomes, DNA, lysosomes

3. What are the main functions of the cytoskeleton in the cell?

- a) it is the external skeleton of cells
- b) it enables the movement of some bacteria
- c) it plays a role in cell division
- d) it enables efficient transport of molecules within cells

4. The cell nucleus:

- a) is the coordinating and regulatory center of the cell
- b) in prokaryotes, it consists of a nuclear membrane, nuclear matrix, and chromatin
- c) contains genetic information
- d) is surrounded by a double nuclear membrane

5. Targeted degradation of selected proteins takes place:

- a) in peroxisomes
- b) in proteasomes
- c) by disjunction
- d) by ubiquitination

6. The cytoplasm:

- a) is the external environment of the cell
- b) can change from sol to gel and vice versa
- c) is semiliquid
- d) proteins and their hydration shells strongly influence its viscosity

7. What is a nucleoid?

- a) the “nucleus” of a prokaryotic cell
- b) the nucleus of a eukaryotic cell
- c) the “nucleus” of a bacterial cell
- d) a circular DNA molecule

8. Which belong among the synthetic centers of the cell?

- a) endoplasmic reticulum
- b) nucleus
- c) lysosome
- d) Golgi apparatus

9. What forms the “nucleus” of a prokaryotic cell?

- a) two circular double-stranded DNA molecules
- b) one circular double-stranded DNA molecule
- c) nucleoid
- d) nucleolus

10. The nucleolus:

- a) is part of a prokaryotic cell
- b) is part of a eukaryotic cell
- c) is the site of tRNA synthesis
- d) contains DNA, RNA, and proteins

11. The nucleus of a eukaryotic cell:

- a) is the coordinating and regulatory center of the cell
- b) contains genetic information
- c) may contain one or more nucleoli
- d) consists of a nuclear membrane, nuclear matrix, and chromatin

12. The nucleus of a eukaryotic cell:

- a) is the coordinating and regulatory center of the cell
- b) contains nuclear pores
- c) cannot have more than one nucleolus
- d) consists of the nuclear membrane, matrix, and nuclear chromosomes

13. The nucleus:

- a) in prokaryotes, the DNA is packaged into nucleosomes by histones
- b) in eukaryotes undergoes cyclic changes during the cell cycle
- c) is the site of translation and polypeptide formation
- d) does not contain RNA

14. Where are mitochondria often localized within the cell?

- a) directly under the plasma membrane
- b) always on the apical side of cells
- c) in the nucleus
- d) at sites with the highest energy demand

15. Which organelles of animal cells are separated from the environment by a double membrane?

- a) mitochondria and nucleolus
- b) mitochondria and chloroplasts
- c) mitochondria and nucleus
- d) mitochondria and endoplasmic reticulum

16. Which statements about the centrosome are correct?

- a) the centrosome consists of two perpendicular centrioles, each composed of nine triplets of tubulin fibers
- b) kinetochore microfilaments bind to the centromere of chromosomes and enable their movement during mitosis
- c) non-kinetochore microtubules participate in moving centrosomes to the poles of the cell
- d) the centrosome is always located in the nucleus during interphase

17. Which statements about the eukaryotic cytoskeleton are correct?

- a) microtubules are involved in vesicle transport and the formation of the mitotic spindle
- b) microfilaments are made of tubulin and ensure chromosome movement
- c) intermediate filaments provide resistance of the cell to tensile and compressive stress
- d) the kinetochore is a protein complex that attaches to the chromosome centromere during division

18. Which statement about mitochondria is true?

- a) 80S ribosomes are assembled in the cytoplasm and then transported into mitochondria
- b) mitochondria have their own genetic information, but it is not expressed – it is only rudimentary
- c) mitochondrial DNA has the same structure as nuclear DNA in eukaryotes
- d) proper mitochondrial function requires interaction of products from both nuclear and mitochondrial genes

19. Which statement correctly describes the types of cytoskeletal fibers?

- a) microtubules – hollow tubes of tubulin, part of the mitotic spindle
- b) microfilaments – thin filaments of actin and myosin, form a contractile system
- c) intermediate filaments – 10 nm non-contractile fibers, increase the mechanical resistance of the cell
- d) microtrabeculae – part of ribosomes, ensure protein synthesis

20. Lysosomes are:

- a) vesicles surrounded by a double membrane containing DNA
- b) vesicles budding from the Golgi apparatus containing hydrolases
- c) structures with oxidative enzymes that detoxify the cell
- d) glycogen storage sites in the cytoplasm

21. Organelles of eukaryotic cells include:

- a) Golgi apparatus
- b) endoplasmic reticulum
- c) mitochondria
- d) lysosomes

22. Microtubules elongate by:

- a) depolymerization
- b) polymerization
- c) addition of tubulin dimers
- d) removal of tubulin dimers

23. Mitochondria arise by:

- a) de novo formation in each daughter cell during cell division
- b) division of existing mitochondria
- c) budding from the nucleus
- d) budding from the endoplasmic reticulum

24. Mitochondria:

- a) are composed of three biological membranes
- b) do not contain DNA
- c) are the site of oxidative phosphorylation
- d) are of maternal origin

25. Mitochondria:

- a) are composed of three biological membranes
- b) contain DNA
- c) are the site of terminal oxidation
- d) are of paternal origin

26. Restoration of the nuclear lamina and nuclear envelope is primarily mediated by:

- a) lamin A
- b) lamin B
- c) lamin C
- d) lamin D

27. The nucleolus:

- a) is a permanent organelle
- b) consists of RNA and proteins
- c) is the site of ribosome production
- d) is the site of proteasome production

28. Peroxisomes are characterized by:

- a) being created de novo from the ER or by division of the original one, containing mainly oxidative enzymes
- b) having a lifespan of decades as permanent cell structures
- c) being the largest organelles of the cell, with protein synthesis as their main function
- d) being encoded only by mitochondrial genes

29. Based on composition, cell organelles are classified as:

- a) membrane-bound
- b) protein-based
- c) composed of proteins and RNA
- d) composed of DNA and RNA

30. Which organelle is characterized by a low internal pH?

- a) lysosome
- b) nucleus
- c) mitochondria
- d) cytoplasm

31. Which organelle is characterized by a high internal pH?

- a) lysosome
- b) nucleus
- c) mitochondrion
- d) cytoplasm

32. Ribosomes:

- a) are not composed of biomembranes
- b) are absent from prokaryotic cells
- c) are the site of transcription
- d) are the site of translation

33. Ribosomes:

- a) are not composed of biomembranes
- b) are absent from eukaryotic cells
- c) are the site of replication
- d) are composed of two protein subunits and rRNA

34. Types of nucleoli include:

- a) compact
- b) nucleolonema type
- c) ring-shaped
- d) nucleolomere type

35. Ribosomes are composed of:

- a) lipopolysaccharides
- b) cholesterol and carbohydrates
- c) ribonucleic acid and proteins
- d) proteins and lipids

36. Cilia and flagella:

- a) are composed of microtubules
- b) cilia provide movement of sperm cells
- c) the flagellum is the motile organelle of some animal cells
- d) are composed only of actin filaments

37. Centrosome and cytoskeleton:

- a) the centrosome consists of two centrioles arranged perpendicular to each other
- b) the cytoskeleton consists of microtubules, microfilaments, and intermediate filaments
- c) the cytoskeleton is composed only of actin proteins
- d) the centrosome has significance only in plant cells

38. Vacuoles:

- a) in plant cells, help maintain turgor
- b) in animal cells, they always have the same size and function
- c) serve in the storage of substances and the degradation of metabolites
- d) arise by nuclear division

39. What is a chromatosome?

- a) a prokaryotic nucleus
- b) a decondensed ribosome
- c) a nucleosome stabilized by histone H1
- d) a structural unit of the chromatin fiber

40. What is the matrisome?

- a) the cohort of genes encoding components of the extracellular matrix
- b) the set of mitochondria in the mother cell
- c) the template for building the cytoskeleton in the cell
- d) the complex of the centrosome and the mitotic spindle microtubules

41. The cell endomembrane system:

- a) includes, for example, membranes of the endoplasmic reticulum, Golgi apparatus, and lysosomes
- b) is present in prokaryotic organisms
- c) is composed mainly of membrane organelles such as peroxisomes and the cytoskeleton
- d) its components can exchange material by vesicular transport

42. Euchromatin:

- a) consists of condensed segments of chromosomes
- b) is transcriptionally inactive chromatin
- c) has a fine, spongy to fibrillar structure
- d) is transcriptionally active

43. Heterochromatin:

- a) consists of condensed segments of chromosomes
- b) is transcriptionally inactive chromatin
- c) has a fine, spongy to fibrillar structure
- d) is transcriptionally active

44. Histone proteins:

- a) belong to the basic (positively charged) proteins
- b) are not found in nuclei of eukaryotic cells
- c) are divided into five main classes
- d) are post-translationally modified

45. The nuclear matrix:

- a) does not contain transcriptosomes and replisomes
- b) represents the most important non-chromatin component of the nucleus
- c) contains enzymes and other proteins required for transcription and replication
- d) lamin A and lamin C contribute to its formation

46. The nuclear envelope:

- a) separates the cell from the extracellular environment
- b) is formed by two biological membranes
- c) its outer membrane is continuous with the endoplasmic reticulum and faces the cytoplasm
- d) its inner membrane is in contact with the endoplasmic reticulum and the cytoplasm

47. One-start model of the 30-nm chromatin fiber is called:

- a) signum
- b) solenoid
- c) chromosome
- d) segment

48. Histone octamer assembly involves:

- a) H1
- b) H3
- c) H4
- d) H8

49. Non-histone proteins associated with the scaffold and nuclear matrix are:

- a) proteins establishing chromosome territories
- b) enzymes that remove torsional stress
- c) proteins that perform transcription
- d) proteins that perform replication

50. The nuclear pore complex consists of:

- a) a central granule
- b) a lateral granule
- c) a diaphragm
- d) the karyotheca

51. Non-histone proteins of the chromosome:

- a) are predominantly regulatory in function
- b) have no effect on the internal organization of the interphase nucleus
- c) include scaffold proteins forming the ultrastructure of chromosomes
- d) protect chromosome territory and govern chromatin loop dynamics within it

52. In which other organelles of eukaryotic cells, besides the nucleus, can genes be found?

- a) mitochondria
- b) Golgi apparatus
- c) chloroplasts
- d) lysosomes

53. The basic components of the cytoskeleton are:

- a) intermediate filaments
- b) centromere
- c) microtubules
- d) microfilaments

54. Globular actin is the fundamental building block of:

- a) intermediate filaments
- b) microtubules
- c) microfilaments
- d) actinoids

55. Nuclear pores:

- a) are located on the inner face of the nuclear envelope
- b) are formed by protein filaments and granules that together make up the pore complex
- c) mediate active transport of substances from the nucleus to the cytoplasm
- d) are found in the nuclear membrane of prokaryotic cells

56. Single-membrane organelles include:

- a) ribosomes and the nucleolus
- b) Golgi apparatus and peroxisomes
- c) lysosomes and endoplasmic reticulum
- d) mitochondria and chloroplasts

57. Nuclear pores:

- a) have a diameter of about 100 μm
- b) are found in the nuclear membrane of prokaryotic cells
- c) mediate active transport from the nucleus to the cytoplasm
- d) are formed from protein filaments and granules

58. Among eukaryotic organelles composed of a single biomembrane are:

- a) chloroplasts, mitochondria
- b) Golgi apparatus
- c) peroxisomes, lysosomes
- d) nucleolus, nucleus

59. Membrane organelles are composed of:

- a) a single membrane, e.g., endoplasmic reticulum
- b) two membranes (nuclear envelope, mitochondria, and chloroplasts)
- c) two membranes, e.g., the Golgi apparatus
- d) a single membrane (nuclear envelope, mitochondria, and chloroplasts)

60. How many biomembranes make up mitochondria?

- a) two
- b) two – the outer envelope and the inner folded membrane
- c) one
- d) two – the outer folded and the inner folded membrane

Answer Key to Model Questions

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|----------|----------|----------|
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| 9. FTTF | 29. TTTF | 49. TTTT |
| 10. FTFT | 30. TFFF | 50. TFTF |
| 11. TTTT | 31. FFTF | 51. TFTT |
| 12. TTFF | 32. TFFT | 52. TFTF |
| 13. FTFF | 33. TFFT | 53. TFTT |
| 14. FFFT | 34. TTTF | 54. FFTF |
| 15. FFTF | 35. FFTF | 55. FTTF |
| 16. TFTF | 36. TFTF | 56. FTTF |
| 17. TFTT | 37. TTFF | 57. FFTT |
| 18. FFFT | 38. TFTF | 58. FTTF |
| 19. TFTF | 39. FFTT | 59. TTFF |
| 20. FTFF | 40. TFFF | 60. TTFF |

5 Cell reproduction

One of the fundamental properties of cells is their ability to reproduce – that is, to divide. This process is crucial for growth, development, tissue regeneration, healing, and repair. In cell biology, three main modes of division are distinguished: **amitosis, mitosis, and meiosis**. Each has a specific mechanism and biological significance.

Amitosis is a rapid, direct form of cell division without the formation of a spindle apparatus and without the typical phases of division. After DNA replication, the nucleus divides directly, often accompanied by constriction of the cytoplasm. It is typical for prokaryotic cells and occurs only rarely in eukaryotes. Because there is no mechanism for accurate chromosome segregation, the genetic stability of daughter cells is not guaranteed. On the other hand, it is a highly efficient means of cell reproduction, and in prokaryotes, it enables rapid colonization of diverse environments, serving as a key survival strategy. Amitosis in prokaryotes is called **binary fission**.

Mitosis is the type of cell division characteristic of somatic cells in multicellular organisms, and is traditionally referred to as indirect cell division. It ensures the precise and equal distribution of genetic information into two genetically identical daughter cells. Following nuclear division (**karyokinesis**), cell division (**cytokinesis**) usually occurs. The key structure driving mitosis is the **mitotic spindle**, a dynamic apparatus composed of microtubules and associated motor proteins. The spindle forms during prophase from the microtubule-organizing centers – centrosomes in animal cells, which contain pairs of centrioles. Its role is to capture chromosomes via kinetochores and direct their movement. The spindle contains three types of microtubules: **kinetochore microtubules**, which attach to centromeres; **polar (non-kinetochore) microtubules**, which overlap and stabilize spindle poles; and **astral microtubules**, which anchor the spindle to the plasma membrane. Coordinated dynamics of these microtubules and the activity of motor proteins, particularly dyneins and kinesins, enable the alignment of chromosomes on the metaphase plate and their precise segregation during anaphase. Proper mitotic spindle function is essential for preserving genomic stability, and defects can lead to chromosome missegregation and **aneuploidy**.

Mitosis proceeds through **prophase, prometaphase, metaphase, anaphase, and telophase**, followed by cytokinesis. The key events are summarized in the table below.

Phase	Main Features	Outcome
Prophase	Chromatin condenses into visible chromosomes. The nucleolus disappears. Centrosomes migrate to opposite poles and begin forming spindle microtubules (especially non-kinetochore MTs). The nuclear envelope remains intact. The Golgi apparatus and the ER are prepared for redistribution.	Compact chromosomes and initial spindle formation.

Prometaphase	Nuclear envelope breakdown (NEBD) via lamin phosphorylation. Envelope fragments remain bound through lamin B to intermediate filaments. Kinetochore MTs attach to sister chromatids. Chromosomes move toward the equator until dynamic equilibrium is achieved.	Chromosomes are attached to the spindle and ready for alignment.
Metaphase	Chromosomes align at the metaphase plate. The spindle assembly checkpoint (SAC) prevents anaphase onset until all kinetochores are properly attached. Activation of APC/C → securin degradation → separase cleaves cohesins at centromeres.	Chromosomes are poised for equal segregation.
Anaphase A	Kinetochore MTs shorten via depolymerization. Sister chromatids are pulled toward opposite poles.	Chromatid separation.
Anaphase B	Non-kinetochore MTs elongate and slide against each other via kinesins. Poles move apart, aided by dyneins and kinesins. Errors may cause lagging chromosomes or nondisjunction.	Increased spindle length and spatial chromosome separation.
Telophase	Chromosomes decondense. Nuclear envelopes reform around chromosome sets. Nucleoli reappear. The spindle disassembles.	Completion of karyokinesis; two nuclei formed.
Cytokinesis	Animals: an actin-myosin contractile ring forms a cleavage furrow at the onset of anaphase, deepening during telophase. Plants: a cell plate (phragmoplast) forms, developing into a new cell wall.	Physical division of the cell → two genetically identical daughter cells.

Meiosis is a reductional division that produces gametes. It reduces the chromosome number from diploid (2n) to haploid (n) and introduces genetic variability through **crossing-over and recombination**. Unlike mitosis, meiosis includes only one round of DNA replication (S phase), followed by two successive divisions: **meiosis I** (reductional division) and **meiosis II** (equational division). The stages of meiosis are summarized in a dedicated table.

Stage	Phase	Main Features	Outcome
Meiosis I (reductional, heterotypic)	Prophase I	<i>Leptotene</i> : onset of chromosome condensation. <i>Zygotene</i> : synapsis of homologous chromosomes, formation of the synaptonemal complex. <i>Pachytene</i> : crossing-over, formation of chiasmata. <i>Diplotene</i> : synaptonemal complex disintegrates, chiasmata remain visible. <i>Diakinesis</i> : maximal condensation, nuclear envelope breakdown (NEBD), disappearance of nucleolus.	Preparation of bivalents for alignment on the metaphase plate.

	Metaphase I	Bivalents align at the metaphase plate. Sister kinetochores are mono-oriented (both facing the same pole).	Ensures correct segregation of homologous chromosomes.
	Anaphase I	Homologous chromosomes separate. Cohesin along arms is degraded, and centromeric cohesin is protected by shugoshin.	Homologs move toward opposite poles.
	Telophase I / Interkinesis	Two haploid cells arise, each containing chromosomes with two chromatids. Interkinesis is brief and lacks DNA replication.	Two haploid cells are prepared for the second division.
Meiosis II (equational, homeotypic)	Prophase II	Chromosomes condense again, spindle forms.	Preparation for the second division.
	Metaphase II	Chromosomes align on the metaphase plate, with sister kinetochores now bi-oriented.	Chromatids are ready for separation.
	Anaphase II	Sister chromatids separate and are pulled toward opposite poles.	Equal segregation of chromatids.
	Telophase II + Cytokinesis	The nuclear envelope reforms, chromosomes decondense, and nucleoli reappear. Cytokinesis divides the cells.	Four genetically distinct haploid cells.

Errors in meiosis, such as nondisjunction (in I or II) and anaphase lag, lead to aneuploidy. Unequal crossing-over alters gene content in chromatids.

The following table presents the differences between mitosis and meiosis.

Difference	Mitosis	Meiosis I & II
Number of divisions	1	2 (reductional and equational)
Result	2 daughter cells	4 gametes
Chromosome number	Maintained (2n)	Reduced to half (n)
Homolog pairing	None	Present, with synapsis and crossing-over
Segregation	Chromatids separate in anaphase	Homologs separate in anaphase I, chromatids in anaphase II
Genetic variability	None (clones)	Present (recombination, segregation)

The **cell cycle** is a sequence of interconnected processes culminating in the formation of daughter cells. It consists of **interphase** (G1, S, and G2 phases) and the **M phase** (mitosis and cytokinesis). **G1 phase**: the cell grows, synthesizes proteins, and prepares for DNA replication. During this period, some cells may exit the cycle and enter the **G0 phase**, characterized by functional differentiation and temporary or permanent withdrawal from further divisions. Differentiation is generally associated with G0, though not all cells in G1 adopt this fate. **S phase**: Semi-conservative DNA replication occurs, producing sister chromatids held together

by cohesins. **G2 phase:** the cell checks and repairs DNA after replication, prepares for mitosis, duplicates centrosomes, and synthesizes tubulin and other spindle components. **M phase:** division of genetic material (mitosis) followed by division of the cytoplasm (cytokinesis).

The accurate progression of the cell cycle is safeguarded by **checkpoints**, positioned at critical phases to ensure fidelity. If errors occur, progression halts to allow repair or, if necessary, apoptosis.

Three main checkpoints ensure the correctness and stability of cellular division (see table).

Checkpoint	Timing	Control Function	Outcome
G1/S checkpoint	End of G1	Assesses cell size, nutrients, and DNA damage	If problems are detected → cycle arrest, repair, or apoptosis
G2/M checkpoint	End of G2	Ensures DNA is fully and correctly replicated, free of damage	Repair mechanisms or block entry into mitosis
Metaphase checkpoint (spindle assembly checkpoint)	During metaphase	Confirms proper attachment of all kinetochores to spindle microtubules	Chromosomes segregate evenly into daughter cells



Model questions

1. Amitosis is:

- a) direct division
- b) indirect division
- c) reduction division
- d) cell differentiation

2. Mitosis is:

- a) direct division
- b) indirect division
- c) reduction division
- d) cell differentiation

3. Meiosis is:

- a) direct division
- b) indirect division
- c) reduction division
- d) cell differentiation

4. Amitosis is the typical mode of division for:

- a) prokaryotic cells
- b) eukaryotic cells
- c) gametes
- d) plants

5. Meiosis is the typical process of producing:

- a) prokaryotic cells
- b) eukaryotic cells
- c) gametes
- d) plants

6. The cell cycle consists of:

- a) interphase and M phase (mitosis)
- b) prophase and metaphase
- c) anaphase and telophase
- d) cytokinesis and karyokinesis

7. Interphase includes:

- a) G1 phase
- b) G2 phase
- c) S phase
- d) M phase

8. The G1 phase is characterized by:

- a) intensive protein synthesis
- b) DNA replication
- c) centrosome duplication
- d) cell differentiation

9. The G0 phase is characterized by:

- a) intensive protein synthesis
- b) DNA replication
- c) centrosome duplication
- d) cell differentiation

10. The G2 phase is characterized by:

- a) intensive protein synthesis
- b) DNA replication
- c) centrosome duplication
- d) cell differentiation

11. The S phase is characterized by:

- a) intensive protein synthesis
- b) DNA replication
- c) centrosome duplication
- d) cell differentiation

12. The main checkpoints of the cell cycle are in:

- a) G1 phase
- b) G2/M phase
- c) S phase
- d) metaphase

13. In mitosis:

- a) two daughter cells are formed, each with the same number of chromosomes as the parent cell
- b) four daughter cells are formed, each with the same number of chromosomes as the parent cell
- c) two daughter cells are formed, each with half the number of chromosomes of the parent cell
- d) four daughter cells are formed, each with half the number of chromosomes of the parent cell

14. In meiosis, the number of chromosomes:

- a) is reduced by half
- b) remains unchanged
- c) doubles
- d) triples

15. Nuclear division occurs by:

- a) the process of cytokinesis
- b) mitosis and meiosis
- c) interphase
- d) karyokinesis

16. The cell cycle:

- a) is a set of processes resulting in the formation of daughter cells
- b) cannot be repeated according to the organism's needs
- c) is the term for DNA synthesis
- d) is the process of chromosome replication

17. Cells can reproduce by:

- a) division
- b) budding
- c) mitosis
- d) meiosis

18. The cell cycle can be divided into:

- a) prophase, prometaphase, metaphase, anaphase, telophase
- b) interphase and cell division
- c) G1 phase, S phase, G2 phase, and M phase
- d) G1 phase, S phase, G2 phase, and interphase

19. Types of cell division (according to the cell cycle) include:

- a) direct division – mitosis
- b) indirect division – mitosis
- c) meiosis
- d) direct division – amitosis

20. Under what conditions does cell division stop?

- a) lack of nutrients
- b) inappropriate temperature
- c) accumulation of harmful substances
- d) unfavorable external conditions

21. A cell has 46 chromosomes during the G1 phase of the cell cycle. How many sister chromatids does it have in prophase I?

- a) 10
- b) 23
- c) 46
- d) 92

22. The cell cycle phases directly related to cell division are:

- a) M phase
- b) G1 and G2 phases
- c) G1 and M phases
- d) S phase

23. Which phase of the cell cycle involves nuclear DNA replication and chromosome duplication?

- a) S phase
- b) synthetic phase
- c) M phase
- d) mitosis

24. Eukaryotic nuclear chromosomes are composed of:

- a) chromatin
- b) DNA
- c) histone proteins
- d) non-histone proteins

25. Interphase of the cell cycle includes:

- a) G1
- b) M
- c) S
- d) G2

26. The correct order of mitotic phases is:

- a) prophase, prometaphase, metaphase, anaphase, telophase
- b) telophase, prophase, prometaphase, metaphase, anaphase
- c) prophase, prometaphase, metaphase, telophase, anaphase
- d) prophase, prometaphase, anaphase, metaphase, telophase

27. In prophase:

- a) chromatin condenses
- b) nuclear envelope disintegrates
- c) chromosomes align at the equatorial plane
- d) the nuclear envelope re-forms

28. The nucleoli disappear in:

- a) prophase
- b) metaphase
- c) anaphase
- d) telophase

29. The early spindle, composed of non-kinetochore microtubules, forms in:

- a) prophase
- b) metaphase
- c) anaphase
- d) telophase

30. Centrosomes separate in:

- a) prophase
- b) metaphase
- c) anaphase
- d) telophase

31. In prometaphase:

- a) chromatin condensation is completed
- b) the nuclear envelope disintegrates
- c) chromosomes align at the equatorial plane
- d) the nuclear envelope re-forms

32. Prometaphase is crucial because:

- a) the nuclear envelope breaks down
- b) non-kinetochore spindle microtubules must properly attach to chromatid cohesins
- c) kinetochore spindle microtubules must properly attach to the kinetochore of both chromatids
- d) each chromosome has two kinetochores located on the centrosome of the chromatid

33. A metaphase chromosome:

- a) is composed of a single chromatid
- b) is composed of two chromatids
- c) has the shape of the letter Y
- d) has the shape of the letter X

34. During metaphase:

- a) the nuclear envelope disintegrates
- b) chromosomes align at the equatorial plane
- c) chromosomes reach maximal condensation
- d) chromosomes decondense

35. Chromosomes are maximally condensed at:

- a) the end of metaphase
- b) the end of anaphase
- c) the end of telophase
- d) the beginning of prophase

36. The anaphase-promoting complex is activated in:

- a) prophase
- b) metaphase
- c) anaphase
- d) telophase

37. In anaphase A:

- a) kinetochore microtubules shorten by depolymerization
- b) non-kinetochore microtubules polymerize
- c) the nucleus divides
- d) the cytoplasm divides

38. In anaphase B:

- a) kinetochore microtubules shorten by depolymerization
- b) non-kinetochore microtubules polymerize
- c) the nucleus divides
- d) the cytoplasm divides

39. During karyokinesis:

- a) kinetochore microtubules shorten by depolymerization
- b) non-kinetochore microtubules polymerize
- c) the nucleus divides
- d) the cytoplasm divides

40. During cytokinesis:

- a) kinetochore microtubules shorten by depolymerization
- b) non-kinetochore microtubules polymerize
- c) the nucleus divides
- d) the cytoplasm divides

41. In telophase, there is:

- a) chromatin condensation
- b) breakdown of the nuclear envelope
- c) alignment of chromosomes at the equatorial plane
- d) formation of a new nucleus

42. During telophase of mitosis:

- a) chromosomes decondense
- b) karyokinesis is completed
- c) new nuclear envelopes form
- d) chromosome condensation occurs

43. Which of the following occurs during telophase?

- a) the actin-myosin contractile ring initiates cleavage furrow formation
- b) sister chromatids separate and are pulled to opposite ends of the cell
- c) microtubules attach to kinetochore proteins on chromosomes
- d) the nuclear membrane breaks down

44. Anaphase is a process that:

- a) can be divided into the anaphase of eukaryotic and prokaryotic cells
- b) occurs after telophase
- c) occurs before telophase
- d) can be subdivided into anaphase A and anaphase B

45. During anaphase of mitosis:

- a) kinetochore microtubules of the spindle depolymerize
- b) single-chromatid chromosomes are pulled toward the centrosomes
- c) kinetochore microtubules shorten by polymerization
- d) two-chromatid chromosomes are pulled toward the centromeres

46. Motor proteins are:

- a) dyneins
- b) kinesins
- c) cohesins
- d) separases

47. Rapid movement of chromosomes toward the centrosomes is aided by:

- a) dyneins
- b) kinesins
- c) polymerization of kinetochore microtubules
- d) depolymerization of kinetochore microtubules

48. If securin degradation fails at the end of metaphase:

- a) separase cleaves cohesins on the inner side of centromeres
- b) separase does not cleave cohesins on the inner side of centromeres
- c) disjunction of sister chromatids occurs
- d) nondisjunction of sister chromatids occurs

49. The protein that protects cohesins from cleavage:

- a) is called separase
- b) is called shugoshin
- c) is removed by ubiquitination
- d) is removed by acetylation

50. What is the site of spindle fiber attachment to sister chromatids during mitosis?

- a) centrosome
- b) centriole
- c) centromere
- d) kinetochore

51. The result of the first meiotic division is cells with:

- a) a haploid number of duplicated chromosomes
- b) a haploid number of non-duplicated chromosomes
- c) a diploid number of duplicated chromosomes
- d) a diploid number of non-duplicated chromosomes

52. The result of the second meiotic division is cells with:

- a) a haploid number of duplicated chromosomes
- b) a haploid number of non-duplicated chromosomes
- c) a diploid number of duplicated chromosomes
- d) a diploid number of non-duplicated chromosomes

53. The outcome of meiosis is the formation of:

- a) one cell
- b) two cells
- c) three cells
- d) four cells

54. In amitosis, the chromosome number:

- a) is preserved
- b) is reduced by half
- c) is doubled
- d) changes randomly depending on cell type

55. How many cells result from the first meiotic division?

- a) 1
- b) 2
- c) 3
- d) 4

**56. In which meiotic phase do homologous chromosomes pair and crossing-over occurs?
And in which phase are chromatids separated?**

- a) in prophase I, and in anaphase II
- b) in anaphase I, and in prophase I
- c) in prophase II, and in anaphase I
- d) in anaphase II, and in prophase II

57. Duplication of chromosomes in meiosis:

- a) does not occur
- b) occurs before the first meiotic division
- c) occurs before the second meiotic division
- d) occurs before both the first and second meiotic divisions

58. The first meiotic division is also called:

- a) reductional
- b) equational
- c) heterotypic
- d) homeotypic

59. The second meiotic division is also called:

- a) reductional
- b) equational
- c) heterotypic
- d) homeotypic

60. The first meiotic division consists of the following phases:

- a) prophase, metaphase, anaphase, telophase
- b) prophase I, metaphase I, anaphase I, telophase I
- c) prophase II, metaphase II, anaphase II, telophase II
- d) proleptotene, leptotene, zygotene, pachytene, diplotene, diakinesis

61. The second meiotic division consists of the following phases:

- a) prophase, metaphase, anaphase, telophase
- b) prophase I, metaphase I, anaphase I, telophase I
- c) prophase II, metaphase II, anaphase II, telophase II
- d) proleptotene, leptotene, zygotene, pachytene, diplotene, diakinesis

62. The stages of prophase of the first meiotic division in correct order are:

- a) leptotene, zygotene, pachytene, diplotene, diakinesis
- b) proleptotene, leptotene, pachytene, diplotene, diakinesis
- c) proleptotene, leptotene, zygotene, diplotene, diakinesis
- d) proleptotene, leptotene, zygotene, pachytene, diplotene, diakinesis

63. Chromosome condensation begins in meiosis at the stage:

- a) proleptotene
- b) zygotene
- c) pachytene
- d) diplotene

64. Synapsis of homologous chromosomes occurs at the stage:

- a) proleptotene
- b) zygotene
- c) pachytene
- d) diplotene

65. Crossing-over occurs at the stage:

- a) proleptotene
- b) zygotene
- c) pachytene
- d) diplotene

66. Recombinant chromatids arise at the stage:

- a) proleptotene
- b) zygotene
- c) pachytene
- d) diplotene

67. Nuclear envelope breakdown and disappearance of the nucleolus occur in meiosis at the stage:

- a) proleptotene
- b) zygotene
- c) pachytene
- d) diakinesis

68. Synaptonemal complexes in meiosis disappear at the stage:

- a) proleptotene
- b) zygotene
- c) pachytene
- d) diplotene

69. Diakinesis is characterized by:

- a) condensation of chromatids
- b) disappearance of chiasmata
- c) breakdown of nuclear envelopes
- d) disappearance of the nucleolus

70. How many chromosome sets does a normal human somatic cell have?

- a) one
- b) two
- c) three
- d) four

71. How many chromosomes does a normal human sperm contain?

- a) 22
- b) 23
- c) 45
- d) 46

72. The main differences between mitosis and amitosis are:

- a) in mitosis, karyokinesis and cytokinesis occur; in amitosis, they do not
- b) mitosis preserves genetic stability, whereas amitosis may disrupt it
- c) mitosis occurs in prokaryotes, amitosis in eukaryotes
- d) amitosis always produces haploid cells

73. During meiosis, the chromosome number:

- a) doubles and is then quartered
- b) is quartered
- c) doubles and is then halved
- d) is halved

74. Nondisjunction is:

- a) an error in the separation of homologous chromosomes or sister chromatids
- b) an error in chromosome migration
- c) deletion of a DNA segment on one chromatid
- d) duplication of a DNA segment on the other chromatid

75. Anaphase lag is:

- a) an error in the separation of homologous chromosomes or sister chromatids
- b) an error in chromosome migration
- c) deletion of a DNA segment on one chromatid
- d) duplication of a DNA segment on the other chromatid

76. Disorders of meiosis include:

- a) senescence
- b) apoptosis
- c) nondisjunction
- d) unequal crossing-over

77. Telomerase is:

- a) the terminal part of a chromosome
- b) an enzyme that elongates telomeres
- c) an enzyme that shortens telomeres
- d) active in embryonic cells

78. Each chromosome in the interphase nucleus has:

- a) its own territory
- b) an arbitrary location
- c) a variable location
- d) a defined position maintained by its association with the nuclear lamina

79. Which cells are haploid?

- a) zygotes
- b) gametes
- c) sex cells
- d) oocytes and sperm

80. What are the identical copies of chromatin held together by cohesin at the centromere called?

- a) histones
- b) nucleosomes
- c) chromatin
- d) sister chromatids

81. Which statements apply to the G₀ phase of the cell cycle?

- a) it represents permanent arrest for all cells
- b) a cell may temporarily exit the cycle
- c) it is associated with cell differentiation
- d) it always occurs immediately after mitosis

82. Which processes characterize the S phase of the cell cycle?

- a) semiconservative DNA replication
- b) synthesis of tubulin and centrosomes
- c) formation of sister chromatids linked by cohesins
- d) division of the cytoplasm

83. What does the G₂/M checkpoint monitor?

- a) uniform attachment of chromosomes to microtubules
- b) accurate DNA replication
- c) DNA damage and its repair
- d) the presence of sufficient nutrients for the cell

84. What does the G1/S checkpoint assess?

- a) cell size
- b) proper attachment of kinetochores to microtubules
- c) availability of nutrients
- d) equal distribution of chromosomes

85. Which statements describe the metaphase (spindle assembly) checkpoint?

- a) it verifies the attachment of all kinetochores to spindle microtubules
- b) it decides on entry into mitosis
- c) it ensures equal segregation of chromosomes
- d) it evaluates cell size and nutrient status

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. TFFF | 30. TFFF | 59. FTFT |
| 2. FTFF | 31. FTFF | 60. FTFF |
| 3. FFTF | 32. TFTF | 61. FFTF |
| 4. TFFF | 33. FTFT | 62. FFFT |
| 5. FFTF | 34. FTTF | 63. TFFF |
| 6. TFFF | 35. TFFF | 64. FTFF |
| 7. TTTF | 36. FTFF | 65. FFTF |
| 8. TFFF | 37. TFFF | 66. FFTF |
| 9. FFFT | 38. FTFF | 67. FFFT |
| 10. FFTF | 39. FFTF | 68. FFFT |
| 11. FTFF | 40. FFFT | 69. FFFT |
| 12. TTFT | 41. FFFT | 70. FTFF |
| 13. TFFF | 42. TTTF | 71. FTFF |
| 14. TFFF | 43. TFFF | 72. TTFF |
| 15. FTFT | 44. FFFT | 73. FFFT |
| 16. TFFF | 45. TTFF | 74. TFFF |
| 17. TTTT | 46. TTFF | 75. FTFF |
| 18. FTTF | 47. TTFT | 76. FFFT |
| 19. FTTF | 48. FTFT | 77. FTFT |
| 20. TTTT | 49. FTTF | 78. TFFT |
| 21. FFFT | 50. FFFT | 79. FTTF |
| 22. TFFF | 51. TFFF | 80. FFFT |
| 23. TTFF | 52. FTFF | 81. FTTF |
| 24. TTTT | 53. FFFT | 82. TFTF |
| 25. TFFT | 54. TFFF | 83. FTTF |
| 26. TFFF | 55. FTFF | 84. TFTF |
| 27. TFFF | 56. TFFF | 85. TFTF |
| 28. TFFF | 57. FTFF | |
| 29. TFFF | 58. TFTF | |

6 Cell and tissue cultures. Assessing basic biological properties of cells

The ***in vitro* cultivation of cells and tissues** is a foundational methodology across biomedical disciplines. It comprises methods that allow long-term maintenance and regulation of cellular life processes outside the organism under precisely defined conditions. This approach enables detailed analysis of cell growth, metabolic pathways, differentiation, and cellular responses to physical, chemical, and biological stimuli. It is also essential for the development of diagnostic methods, testing drug efficacy and toxicity, studying disease mechanisms, and advancing tissue engineering and regenerative medicine.

Primary culture – derived directly from tissue after enzymatic or mechanical dissociation. It may be short-term (lasting hours to days) or long-term (lasting more than 10 days). **Cell line** – established from a primary culture by passaging; has a finite lifespan (e.g., human fibroblasts ~25 passages). **Permanent (immortalized) line** – has unlimited proliferative capacity, arising spontaneously (e.g., HeLa) or via viral transformation.

Biology of cultured cells

Cell behavior ***in vitro*** differs markedly from that ***in vivo***. Phenotype shifts occur, including changes in cell shape, altered metabolism, and modifications in interactions with neighboring cells and the extracellular matrix (ECM). In classical **2D cultures**, spatial organization and tissue heterogeneity are lacking, which limits cell-cell and cell-ECM contacts and often leads to the loss of specialized functions and dedifferentiation. These limitations led to **3D culture systems** (cell aggregates, spheroids, organoids, or growth on biocompatible matrices/scaffolds), which better recapitulate *in vivo* architecture, support physiological differentiation, preserve signaling dynamics, and sustain functional ECM interactions.

By growth mode and interaction with the culture surface, cultures are **adherent** or **suspension**.

Adherent cultures attach to substrates via **integrins** (binding collagen, fibronectin, and laminin), **cadherins**, **cell adhesion molecules (CAMs)**, and transmembrane proteoglycans. Culture ware is often coated with ECM components to promote adhesion. Adherent cells form a monolayer; after reaching **80–90% confluence**, they require passaging (enzymatic detachment and redistribution to new vessels). Primary cells and many lines have a finite division capacity (**Hayflick limit**): after ~40–60 divisions, proliferation ceases due to telomere shortening and the onset of replicative senescence.

Suspension cultures grow freely in a medium without adhesion (typical of hematopoietic cells, such as lymphocytes or leukemic lines, or some tumor cells). Continuous agitation is often required to prevent aggregation and ensure uniform access to nutrients and oxygen. Advantages include high cell density and straightforward processing of large volumes (useful in

biotechnology and pharmaceutical production). Disadvantages include limited morphological assessment and lower physiological relevance compared with adherent cultures.

Regulation of cellular behavior in culture is predominantly **autocrine and paracrine**, so media are supplemented with hormones and growth factors. Metabolically, cells preferentially use **aerobic glycolysis** (the Warburg effect) with high glucose consumption. The Krebs cycle remains active, and nitrogen/carbon is supplied chiefly by glutamine or more stable derivatives, which are added to culture media.

Success is influenced by the time of tissue/cell collection and subsequently depends on the following factors: **Sterility** – work in a laminar flow hood; sterilize instruments and solutions; use of antibiotics and/or antimycotics as needed. **Temperature** – for most animal cells ~37 °C. **pH and CO₂** – maintained at pH 7.1–7.4 using buffers and CO₂ incubators. **Osmotic pressure** – approximately **7.6 atm**, tolerance ±10%. Appropriate **culture medium** – water, salts, amino acids, vitamins, carbohydrates, and often serum with growth factors. **Substrate** – the surface of the vessel for adherent cultures and the medium itself for suspension cultures.

Growth of cultured cells is commonly depicted by a growth curve with four characteristic phases. The first phase is the lag phase, also known as the adaptation period. Immediately after seeding cells into fresh culture medium, cells adapt to the new conditions. In adherent cultures, this includes adhesion to the culture surface, cytoskeletal reorganization, and activation of metabolic pathways. Mitotic activity is minimal, so cell number remains essentially unchanged.

The **logarithmic (log) phase** follows, during which cells divide at the maximal rate. Growth is exponential – in ideal conditions, the population doubles at regular intervals corresponding to the generation time. In this phase, cells are most metabolically active, display typical morphology, and respond most representatively to stimuli. For these reasons, the log phase is optimal for experimental interventions such as cytotoxicity testing, transfections, or sampling.

The **stationary phase (plateau)** arises when cells reach the limit of available growth area or deplete the medium nutrients. In adherent cells, **contact inhibition** plays a major role – once a monolayer forms, cells stop dividing even though they remain metabolically active. Growth therefore slows, and the population reaches a stable size.

The last is the **decline (death) phase**, in which cells gradually die due to nutrient depletion, accumulation of toxic metabolites, or aging. Signs of senescence and apoptosis appear, and at advanced stages, also necrosis. The culture loses experimental value and must be renewed by passaging or re-established from a primary source.

Applications of cell and tissue cultures

Cell and tissue cultures are integral to modern biomedical research and clinical practice. In **virology**, they enable the identification of viruses, the study of cytopathic effects, and are key

tools in vaccine development and production. **Pharmacotoxicology** utilizes cultures to assess the cytotoxicity of new chemicals and drugs, elucidate their mechanisms of action, and develop ADMET models (absorption, distribution, metabolism, excretion, and toxicity) that underpin preclinical safety and efficacy assessments. In **human genetics**, cultures provide a critical source of cells for karyotyping, fluorescence in situ hybridization (FISH), DNA analyses, and various biochemical tests. **Oncological research** utilizes patient-derived cell lines to assess sensitivity or resistance to cytostatics, to model tumor biology, and to explore novel therapeutic strategies. In **reproductive medicine**, cell culture is applied in *in vitro* fertilization and subsequent embryo culture, forming the basis of assisted reproduction. **Tissue engineering and regenerative medicine** are developing rapidly, using cultures to generate skin, cartilage, bone, and soft-tissue replacements with various stem cell types, including induced pluripotent stem cells, opening avenues to personalized disease models and new therapies.

Assessing basic biological properties of cells

Assessing fundamental cellular properties is essential for both experimental use and clinical applications. Key parameters include **cell viability** and **cytotoxicity**, which determine whether cultured cells survive and to what extent they are damaged. Several approaches based on distinct biological principles are used.

Membrane integrity assays evaluate preservation of selective membrane permeability. The classic **trypan blue exclusion test** distinguishes between viable cells (cells with intact membranes) and dead cells (cells with compromised membranes), as viable cells remain unstained, while dead cells stain blue. Limitations include subjectivity and lower sensitivity, which can be mitigated by **fluorescent dyes** (e.g., **propidium iodide** or **SYTOX Green**) that allow more precise viability assessment.

Metabolic assays assess mitochondrial enzyme activity as a proxy for vital functions. The widely used **MTT assay** measures the reduction of yellow tetrazolium to insoluble purple formazan, which is then solubilized and quantified spectrophotometrically. An alternative is the **Alamar Blue (resazurin) assay**, in which viable cells reduce resazurin to the fluorescent compound resorufin, allowing for sensitive, non-invasive, and repeatable real-time monitoring of metabolic activity.

A third approach detects **release of intracellular markers** into the medium due to membrane damage. The most common is the **LDH assay**, which measures lactate dehydrogenase released from damaged cells, using colorimetric or fluorometric readouts.

In recent years, modern technologies have enabled more comprehensive and precise analyses. **Flow cytometry** offers a rapid, quantitative, and multiparameter assessment of viability, apoptosis, and the cell cycle at the single-cell level. **Live-cell confocal microscopy** provides detailed information on dynamic changes in morphology, membrane potential, and apoptotic processes in real-time. **Impedance-based systems** (e.g., **xCELLigence**, **ECIS**) noninvasively

track growth, proliferation, and cytotoxic effects via changes in electrical resistance, allowing for continuous monitoring without the need for staining.

By combining classical and modern methods, a comprehensive picture of cell status, proliferation, metabolic activity, and responses to physical, chemical, or biological stimuli can be obtained. Such data are essential not only for the accurate interpretation of experimental results but also for their translation into clinical and biotechnological applications, including the development of new drugs, biomaterials, and personalized therapeutic strategies.



Model questions

1. Cell adhesion to the substrate is mediated mainly by:

- a) integrins
- b) cadherins
- c) CAMs
- d) a surface coated with collagen/fibronectin improves adhesion

2. A cell culture:

- a) is a population of cells that proliferates *in vitro*
- b) is a population of cells that proliferates *in vivo*
- c) is organized as a tissue
- d) is not organized as a tissue

3. A cell culture:

- a) is a population of cells that proliferate *in vitro* and are not organized as a tissue
- b) can be adherent or suspension
- c) is used in *in vitro* fertilization
- d) can be depicted by a Gaussian curve

4. A cell line:

- a) is organized as a tissue
- b) is a simplified, miniaturized organ *in vitro*
- c) is a population arising from the repeated passaging of cells from a primary culture
- d) is used in biomedical research

5. Cell cultures are used:

- a) in pharmacotoxicology
- b) in *in vitro* fertilization
- c) in human genetics
- d) in tissue engineering

6. A cell culture can be established from:

- a) bacteria
- b) prokaryotes
- c) human tissues
- d) eukaryotes

7. Explant culture:

- a) is a cultivation technique that uses various enzymes
- b) is an organotypic culture technique from a small piece of tissue
- c) is transferring a small number of cultured cells to a new vessel
- d) arrests cell growth

8. Immortalized cultures:

- a) can also be obtained by transforming cells with various bacteria
- b) are used in cytogenetic diagnostics
- c) are not used in *in vitro* fertilization
- d) are used in toxicology

9. Immortalized cultures:

- a) can be obtained by transforming cells with various viruses
- b) are used in toxicology
- c) are used to analyze the genotoxicity of selected substances
- d) are used to culture fertilized oocytes

10. Short-term cultures:

- a) are maintained for 24 hours or at most a few days
- b) are not used in cytogenetics
- c) are maintained for more than 10 days
- d) may show high spontaneous mitotic activity (e.g., bone marrow cells)

11. Which of the following are important uses of human cell culture?

- a) preparation of karyological specimens for cytogenetic analysis
- b) testing tumor cell sensitivity to cytostatics
- c) *in vitro* fertilization (“test-tube fertilization”)
- d) preparation of DNA vaccines in bacteria

12. Which statement best describes a primary cell culture?

- a) it arises directly from tissue by mechanical or enzymatic dissociation of cells
- b) it is a cell line with unlimited proliferative capacity
- c) it is always non-adherent
- d) it originates exclusively from suspension cells

13. Which factor most strongly affects successful initial cell adhesion?

- a) the presence of antibiotics in the medium
- b) the composition of the medium and the type of vessel surface
- c) centrifugation time after sampling
- d) the shape of the culture flask

14. To ensure sterility while handling cell cultures, use:

- a) a laminar-flow hood with a UV lamp and laminar air flow
- b) a water bath
- c) a microscope with a heated stage
- d) ultrasonic sterilization of medium

15. To prevent contamination of a cell culture, use:

- a) fetal bovine serum
- b) streptomycin, penicillin
- c) amphotericin
- d) trypsin

16. Optimal conditions for animal cell culture include:

- a) ~37 °C
- b) pH ~7.1–7.4
- c) 0% CO₂ in the incubator
- d) osmolality within a tolerance of about $\pm 10\%$

17. Organoids:

- a) arrest cell growth
- b) are used to create cellular disease models
- c) have applications in personalized medicine
- d) are simplified, miniaturized versions of organs cultured in 3D *in vitro*

18. Passaging cells:

- a) prevents senescence of cultured cells
- b) is transferring a small number of cultured cells to a new culture vessel
- c) arrests cell growth
- d) is caused by frequent medium changes

19. A permanent cell line:

- a) is a population that can divide indefinitely
- b) may originate, for example, from a biopsy of malignant tumor tissue
- c) is a simplified, miniaturized organ *in vitro*
- d) is also called an immortalized cell line

20. The plateau phase of cell culture growth:

- a) is also called the stationary phase
- b) is when cells adapt to *in vitro* conditions
- c) is the phase of rapid cell multiplication
- d) is when cells remain viable but no longer divide

21. Tissue culture:

- a) proliferating cells or tissues in culture medium outside the organism
- b) a population of cells that proliferates *in vivo*
- c) arrests cell growth
- d) is organized as a tissue

22. Which molecules mediate cell adhesion to the substrate?

- a) integrins
- b) cadherins
- c) cytokines
- d) cell adhesion molecules (CAMs)

23. Which statements about the Hayflick limit are correct?

- a) it applies to primary cells and cell lines
- b) it is a finite number of divisions due to telomere shortening
- c) it describes the unlimited division of immortalized lines
- d) it corresponds to roughly 40–60 cell division cycles

24. Regarding contact inhibition of cell growth:

- a) cell division stops once a monolayer forms and cells contact each other
- b) it is typical of normal adherent cells in culture
- c) cancer cells often lose this mechanism
- d) in suspension cultures, arrest is primarily due to cell-cell contact

25. For maintenance of suspension cultures, one must ensure:

- a) continuous agitation
- b) uniform access to nutrients and oxygen
- c) collagen coating of the vessel bottom
- d) prevention of cell aggregation

26. Metabolic features typical of cultured cells include:

- a) preferential use of aerobic glycolysis (Warburg effect)
- b) high glucose consumption
- c) an inactive Krebs cycle
- d) use of glutamine or its derivatives as sources of nitrogen and carbon

27. The log phase of cell culture growth:

- a) is when cells remain viable but no longer divide
- b) is the phase of rapid proliferation
- c) is when cells adapt to *in vitro* conditions
- d) is also called the stationary phase

28. Vital staining:

- a) is used for staining native (unfixed) preparations
- b) is used after staining fixed preparations
- c) allows assessment of cell viability
- d) enables long-term archiving of preparations

29. The Alamar Blue test is based on:

- a) reduction of resazurin to fluorescent resorufin in metabolically active cells
- b) degradation of LDH upon membrane damage
- c) uptake of trypan blue by live cells
- d) nonspecific reduction of formazan crystals

30. How does the MTT assay work?

- a) a qualitative membrane-integrity test to distinguish live and dead cells
- b) a sensitive test with a linear relationship between cell activity and absorbance
- c) a mitochondrial reductase reduces MTT to insoluble formazan crystals
- d) a qualitative test based on nonspecific enzymatic reduction of resazurin

31. The main difference between the LDH assay and trypan blue staining is that:

- a) the LDH assay detects the release of an intracellular enzyme upon membrane damage
- b) trypan blue assesses mitochondrial metabolic activity
- c) the LDH assay is fluorescent or colorimetric, whereas trypan blue is only colorimetric
- d) trypan blue yields quantitative results independent of subjectivity

32. LDH test:

- a) enables the determination of the extent of cell death
- b) is used to measure leakage markers from the cytoplasm
- c) determines the kinetics of cell proliferation
- d) is a colorimetric or fluorometric test

33. Why use a combination of cytotoxicity assays?

- a) different assays probe distinct aspects of viability (membrane, metabolism, leakage)
- b) each single assay is perfectly accurate and sufficient on its own
- c) combining assays increases reliability and objectivity
- d) most assays are cheap, so they are run all at once

34. Which methods are metabolic viability assays?

- a) MTT test
- b) LDH test
- c) Alamar Blue test
- d) Trypan blue exclusion test

35. Advantages of the Alamar Blue test over MTT:

- a) non-invasive
- b) allows repeated real-time measurements
- c) based on a fluorescent signal
- d) requires killing the cells

36. What does the LDH test detect?

- a) activity of mitochondrial enzymes
- b) cellular DNA content
- c) lactate dehydrogenase released from damaged cells
- d) apoptotic membrane fragments

37. Modern technologies for assessing cell viability:

- a) flow cytometry
- b) live-cell confocal microscopy
- c) impedance systems
- d) Western blot

38. Advantages of impedance systems:

- a) enable non-invasive, continuous monitoring of cell growth
- b) require vital dyes
- c) are based on changes in electrical resistance
- d) provide only one-time readouts

39. What does live-cell confocal microscopy reveal?

- a) dynamic changes in cell morphology
- b) changes in membrane potential
- c) detection of apoptotic processes
- d) only endpoint images of fixed cells

40. Why combine multiple viability assays?

- a) each assay captures a different aspect of viability
- b) to obtain a more comprehensive picture of cell status
- c) to reduce false negatives/positives
- d) because single assays cannot be used alone

41. What is often added to media to support growth and viability?

- a) hormones
- b) growth factors
- c) antibiotics
- d) vitamins and trace elements

42. Main readout in metabolic assays:

- a) membrane integrity
- b) activity of mitochondrial enzymes
- c) intracellular Ca^{2+} level
- d) number of nuclei

43. Limits of the trypan blue exclusion test:

- a) cannot distinguish apoptotic from live cells
- b) subjective evaluation
- c) requires expensive instruments
- d) lower sensitivity than fluorescent methods

44. Flow cytometry enables:

- a) rapid quantitative analysis
- b) multiparameter measurements on single cells
- c) assessment of viability and apoptosis
- d) monitoring growth of whole tissue structures

45. Methods for label-free continuous monitoring of proliferation:

- a) impedance systems
- b) trypan blue exclusion test
- c) confocal microscopy
- d) LDH test

46. Hallmarks of 3D culture systems vs. classic 2D:

- a) better mimic the in vivo environment
- b) cannot support differentiation
- c) are always short-term
- d) do not allow ECM interactions

47. What limits the number of divisions in primary cells and many lines?

- a) apoptosis
- b) the Hayflick limit
- c) autophagy
- d) the Warburg effect

48. Typical of suspension cell cultures:

- a) require adhesion to the vessel surface
- b) grow freely in the medium
- c) do not need nutrients and oxygen
- d) used only in genetics

49. The main reason for the passaging of adherent cells is:

- a) to increase the medium pH
- b) reaching 80–90% confluence
- c) removal of old cells via apoptosis
- d) inhibition of cell-ECM contact

50. Metabolite most often added as a nitrogen/carbon source:

- a) glucose
- b) alanine
- c) glutamine
- d) lactate

51. What does the lag phase of a growth curve represent?

- a) maximal mitotic activity
- b) cellular adaptation with low mitotic activity
- c) onset of degradation and senescence
- d) arrest due to contact inhibition

52. Feature of the Warburg effect:

- a) predominance of aerobic glycolysis despite oxygen presence
- b) complete blockade of the Krebs cycle
- c) fatty acids as the main energy source
- d) activated only in suspension cultures

53. Which assay measures mitochondrial enzyme activity via tetrazolium reduction?

- a) LDH test
- b) MTT test
- c) trypan blue
- d) PCR test

54. A risk when working with permanent cell lines:

- a) short culture lifespan
- b) spontaneous differentiation into specialized cells
- c) accumulation of genetic changes and divergence from the original phenotype
- d) inability to grow at a larger scale

55. Which approach tracks changes in the electrical resistance of a cell layer during growth and proliferation?

- a) Western blot
- b) MTT test
- c) FISH
- d) impedance systems

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. TFFT | 20. TFFT | 39. TTTF |
| 2. TFFT | 21. TFFT | 40. TTTF |
| 3. TTTF | 22. TTFT | 41. TTFT |
| 4. FFTT | 23. TTFT | 42. FTFF |
| 5. TTTT | 24. TTTF | 43. TTFT |
| 6. TTTT | 25. TTFT | 44. TTTF |
| 7. FTFF | 26. TTFT | 45. TFFF |
| 8. FFTT | 27. FTFF | 46. TFFF |
| 9. TTTF | 28. TFTF | 47. FTFF |
| 10. TFFT | 29. TFFF | 48. FTFF |
| 11. TTTF | 30. FFTF | 49. FTFF |
| 12. TFFF | 31. TFTF | 50. FFTF |
| 13. FTFF | 32. TTFT | 51. FTFF |
| 14. TFFF | 33. TFTF | 52. TFFF |
| 15. FTTF | 34. TFTF | 53. FTFF |
| 16. TTFT | 35. TTTF | 54. FFTF |
| 17. FTTT | 36. FFTF | 55. FFFT |
| 18. FTFF | 37. TTTF | |
| 19. TTFT | 38. TFTF | |

7 Cell death

Apoptosis is a genetically regulated process of programmed cell death, and it is a key mechanism for maintaining tissue homeostasis. It occurs physiologically during embryonic development, tissue renewal, and regeneration, and serves as a protective mechanism against cells that are damaged, dysfunctional, or at risk of malignant transformation. Unlike **necrosis**, which is a pathological, inflammation-provoking form of cell death associated with autolysis, apoptosis is tightly regulated and does not trigger an inflammatory response. During apoptosis, the cell gradually shrinks; chromatin condenses and the nucleus shrinks (**pyknosis**), and the cytoplasm becomes denser. The plasma membrane remains intact, but small membrane-bound fragments, **apoptotic bodies**, bud off and are rapidly engulfed by phagocytes or neighboring cells.

Functions of apoptosis can be summarized into two. First, **physiological turnover**: life-span limitation, developmental sculpting, and immune system selection during embryogenesis and maturation. Second, **protection** of the organism, including the elimination of cells threatened by malignancy or foreign elements.

Apoptosis is essential for the proper remodeling of structures in the embryonic stage. An imbalance between proliferation and apoptosis during development leads to **congenital malformations**.

Apoptosis is initiated via two principal pathways. The **extrinsic (death-receptor) pathway** is triggered by ligand binding to death receptors, such as Fas or TNF receptors, followed by receptor oligomerization, the recruitment of adaptor proteins (e.g., FADD), and the activation of initiator **caspase-8**. This, in turn, activates effector caspases (**3, 6, 7**), resulting in the morphological and biochemical features characteristic of apoptosis.

The intrinsic (mitochondrial) pathway: Initiated by intracellular damage (DNA lesions, oxidative stress, hypoxia, telomere erosion). **p53** acts as a “guardian of the genome,” inducing pro-apoptotic Bcl-2 family members (**Bax, Bak**), which permeabilize the mitochondrial outer membrane and release **cytochrome c**. Cytochrome c binds **Apaf-1** in the presence of ATP to form the **apoptosome**, which activates initiator **caspase-9**, converging on the same effector caspases as above.

A distinct mechanism is **ER-stress-induced apoptosis**, which occurs when misfolded proteins or toxic metabolites accumulate. In rodents, this process involves procaspase-12; in humans, analogous roles are primarily played by caspase-4 and caspase-5.

Despite different initiating signals, all apoptotic pathways converge on a common effector cascade, culminating in DNA fragmentation, chromatin condensation, the formation of apoptotic bodies, and their phagocytic clearance, all without provoking inflammation.

Autophagy is a process in which the cell degrades and recycles its own damaged or unnecessary organelles and macromolecules. It is primarily a survival mechanism activated by nutrient deprivation, stress conditions, or the accumulation of aberrant proteins. A double-membrane vesicle, known as the **autophagosome**, forms around the cargo and then fuses with a lysosome to form an **autolysosome**, where degradation occurs through the action of hydrolytic enzymes. The recycled products (amino acids, fatty acids, and nucleotides) are reintroduced into cellular metabolism. Autophagy maintains homeostasis, defends against intracellular pathogens, and helps regulate the cell cycle. Its dysregulation is linked to neurodegenerative diseases (e.g., Alzheimer's and Parkinson's), cancer, and metabolic disorders.

Necrosis is a passive and invariably pathological process of cell death that results from severe injury. Unlike apoptosis, it is not a programmed mechanism, but rather a consequence of profound stress, energy failure, or exposure to exogenous harmful agents. Typical features include cellular swelling, membrane rupture, and the release of intracellular contents, which elicit an inflammatory response. Triggers include ischemia, hypoxia, toxins, infections, autoimmune injury, and mechanical trauma.

In addition to classical apoptosis and necrosis, several other types of regulated cell death have been identified in recent decades, which differ in mechanism, morphological manifestations, and biological significance. Selected ones are summarized in the following table.

Type of cell death	Mechanism	Morphological features	Examples / Significance
Pyroptosis	Inflammasome activation → caspase-1/4/5/11 → IL-1 β , IL-18 release	Cell swelling, membrane pores, lysis, inflammation	Host defense against intracellular pathogens (e.g., <i>Salmonella</i> , <i>Shigella</i>)
Necroptosis	RIPK1/RIPK3 activation, MLKL phosphorylation → membrane permeabilization	Swelling and lysis with content release; inflammatory	Alternative death when apoptosis is blocked; immunologic signaling
Ferroptosis	Lipid peroxide accumulation, GPX4 failure; Fe ²⁺ -dependent oxidative stress	Smaller, dense mitochondria; lacks classic apoptotic features	Neurodegeneration, ischemia-reperfusion injury
Anoikis	Loss of cell-ECM attachment → activation of apoptotic pathways	Apoptotic morphology (pyknosis, apoptotic bodies)	Prevents ectopic colonization; resistance supports metastasis
NETosis	Neutrophil activation → degranulation and extrusion of nuclear DNA + proteins → NET formation	Nuclear breakdown, membrane disintegration, release of chromatin, and antimicrobial proteins	Antimicrobial defense (bacteria, fungi, viruses); pathological in autoimmunity (e.g., lupus, RA)



Model questions

1. What are the fundamental mechanisms that induce apoptosis?

- a) via the mitochondrial pathway
- b) a stress route via the Golgi apparatus
- c) via the death-receptor pathway
- d) via a lysosomal pathway

2. What is the function of apoptosis in the organism?

- a) to promote muscle growth
- b) to protect cells from UV radiation
- c) to remove damaged or unnecessary cells
- d) to produce energy for the cell

3. Apoptosis is:

- a) programmed cell death
- b) immunologically “silent” cell death
- c) another name for necrosis
- d) a physiological form of cell death

4. Apoptosis plays a key role in:

- a) preventing tumor formation
- b) ATP production for cellular metabolism
- c) protein synthesis
- d) accumulation of cellular waste

5. Apoptosis is primarily characterized by:

- a) cell shrinkage and DNA fragmentation
- b) uncontrolled cellular swelling
- c) inflammation and immune activation
- d) phagocytosis of damaged organelles

6. Apoptosis is crucial during the development of the:

- a) heart
- b) digits (fingers)
- c) intestine
- d) brain

7. Apoptosis differs from necrosis in that:

- a) apoptosis is not accompanied by inflammation
- b) necrosis involves phagocytosis
- c) apoptosis is not regulated
- d) necrosis is often caused by trauma

8. Autophagy is typically activated in response to:

- a) nutrient deprivation
- b) energy surplus
- c) the presence of apoptotic cells
- d) elevated oxygen levels

9. Autophagy can lead to cell death if:

- a) it becomes excessively active
- b) cellular ATP levels are high
- c) caspase activity is low
- d) lysosomes are inactive

10. Autophagy serves to:

- a) recycle cellular components
- b) initiate cell division
- c) randomly degrade proteins
- d) create energy stores outside the cell

11. Principal functions of apoptosis:

- a) programmed cell death during cellular selection
- b) regulated cell death protecting multicellular organisms from risky cells
- c) programmed cell death at the end of a cell's "life span"
- d) programmed cell death during embryogenesis

12. Caspases:

- a) may be non-functional enzymes
- b) are proteases
- c) exist in the cytoplasm as inactive procaspases
- d) can be active

13. Key proteins involved in initiating apoptosis include:

- a) caspases
- b) ATG proteins
- c) p53
- d) chaperones

14. Which structure is responsible for the actual degradation of cellular contents during autophagy?

- a) autolysosome
- b) phagosome
- c) peroxisome
- d) autophagosome

15. Which of the following pathways is associated with apoptosis?

- a) gluconeogenesis
- b) the MAPK pathway
- c) the death-receptor pathway
- d) glycolysis

16. Which morphological features are typical of necrosis?

- a) cell shrinkage and nuclear fragmentation
- b) cell swelling and membrane rupture
- c) formation of double-membrane vacuoles
- d) organelle breakdown with release of cellular contents

17. Which statements characterize extrinsic activation of apoptosis?

- a) triggered by ligand binding to a death receptor (e.g., Fas, TNF)
- b) caused primarily by DNA mutations and telomere shortening
- c) activation of initiator caspase-8 occurs
- d) triggered by cytochrome-c release from mitochondria

18. Which statements about autophagy are correct?

- a) it is a recycling process for cellular organelles and macromolecules
- b) it proceeds via autophagosome formation and fusion with lysosomes
- c) it is always accompanied by a marked inflammatory response
- d) it is a hallmark of pathological cell death

19. Which statements about necrosis are correct?

- a) it is programmed cell death governed by caspases
- b) it is associated with an inflammatory response in the surrounding tissue
- c) it arises from injury such as hypoxia or toxins
- d) it is characterized by the formation of apoptotic bodies

20. Major caspases include:

- a) caspase-3
- b) caspase-8
- c) caspase-9
- d) caspase-6

21. Principal proteins of apoptosis include:

- a) caspases
- b) albumin
- c) Apaf-1
- d) Bax

22. Morphological manifestations typical of apoptosis:

- a) dense aggregation of chromatin at the nuclear envelope
- b) cell shrinkage
- c) formation of apoptotic bodies
- d) biogenesis of new mitochondria

23. Unlike apoptosis, necrosis:

- a) often provokes inflammation
- b) is a regulated form of cell death
- c) results in apoptotic body formation
- d) is the consequence of unplanned cellular injury

24. In contrast to apoptosis, necrosis is:

- a) a rapid and uncontrolled process
- b) associated with phagocytosis of whole cells
- c) energy-dependent
- d) typically linked to immune activation

25. Necrosis is:

- a) a random form of cell death
- b) a process triggered by external injury
- c) similar to apoptosis
- d) a controlled mechanism of cell death

26. Necrosis can be triggered by:

- a) extreme temperature changes
- b) nutrient deficiency
- c) genetic mutations
- d) toxic substances

27. During apoptosis, the plasma membrane:

- a) remains intact until late stages
- b) ruptures immediately
- c) incorporates more lipids
- d) fuses with neighboring cell membranes

28. In the autophagy pathway, damaged organelles are delivered to:

- a) the nucleus
- b) lysosomes
- c) the Golgi apparatus
- d) mitochondria

29. During embryogenesis, apoptosis is essential for:

- a) remodeling of cardiac septa
- b) activating lysosomes in gut development
- c) shaping of limb digits
- d) transfer of genetic information between neurocytes

30. In necrosis, cells:

- a) lose membrane integrity
- b) undergo controlled disassembly
- c) fragment into apoptotic bodies
- d) release their contents, damaging the surrounding tissue

31. The primary difference between apoptosis and autophagy is that:

- a) apoptosis culminates in cell death, whereas autophagy can promote cell survival
- b) autophagy always triggers inflammation
- c) apoptosis occurs only during embryonic development
- d) autophagy does not involve lysosomes

32. Typical features of necrosis include:

- a) cell swelling
- b) cell shrinkage
- c) membrane rupture
- d) release of cellular contents into surrounding tissues

33. Which statements about pyroptosis are correct?

- a) it is mediated by the inflammasome
- b) it activates caspases 1, 4, 5, and 11
- c) it proceeds without an inflammatory response
- d) it is important for defense against intracellular pathogens

34. What characterizes necroptosis?

- a) activation of the kinases RIPK1 and RIPK3
- b) phosphorylation of the protein MLKL
- c) a complete absence of inflammatory response
- d) an alternative death route when apoptosis is blocked

35. Which morphological changes are typical of ferroptosis?

- a) shrinkage of mitochondria
- b) increased density of the mitochondrial matrix
- c) DNA fragmentation as in apoptosis
- d) absence of classic apoptotic features

36. What characterizes anoikis?

- a) it is triggered by loss of cell contact with the extracellular matrix
- b) it proceeds via activation of apoptotic pathways
- c) it is a variant of necrosis
- d) its suppression promotes metastasis

37. What happens during NETosis?

- a) neutrophils are activated
- b) nuclear DNA and proteins are released
- c) extracellular traps (NETs) are formed
- d) the process occurs without disintegration of the cell membrane

38. What is typical of the extrinsic pathway of apoptosis?

- a) activation of death receptors (Fas, TNF receptors)
- b) receptor oligomerization and recruitment of adaptor proteins (FADD)
- c) activation of initiator caspase-8
- d) release of cytochrome c from mitochondria

39. Which injuries can activate the intrinsic pathway of apoptosis?

- a) DNA mutations
- b) oxidative stress
- c) hypoxia
- d) contact with a TNF receptor

40. What role does p53 play in the intrinsic pathway of apoptosis?

- a) it acts as the “guardian of the genome”
- b) it activates transcription of pro-apoptotic Bcl-2 family members
- c) it stabilizes the mitochondrial membrane
- d) it prevents mitochondrial permeabilization

41. What is the apoptosome, and what is its function?

- a) a complex of cytochrome c, Apaf-1, and ATP
- b) it activates initiator caspase-9
- c) it is the result of death-receptor activation
- d) it controls the expression of Bcl-2 genes

42. Which caspases are effector caspases?

- a) caspase-3
- b) caspase-6
- c) caspase-7
- d) caspase-9

43. What is typical of ER-stress-induced apoptosis?

- a) accumulation of misfolded proteins
- b) activation of procaspase-12 (in rodents)
- c) in humans, mainly caspase-4 and caspase-5 are involved
- d) activation of TNF receptors

44. What are the resulting morphological hallmarks of apoptosis?

- a) DNA fragmentation
- b) chromatin condensation
- c) formation of apoptotic bodies
- d) extensive tissue inflammation

45. What do all apoptotic pathways have in common?

- a) they activate a common effector caspase cascade
- b) they end with the formation of apoptotic bodies
- c) they trigger an inflammatory response
- d) they lead to DNA fragmentation and chromatin condensation

46. Which feature distinguishes apoptosis from necrosis?

- a) DNA fragmentation and formation of apoptotic bodies
- b) cell swelling and membrane rupture
- c) it always evokes an inflammatory response
- d) it is not genetically programmed

47. Which protein plays a key role in the intrinsic apoptotic pathway?

- a) p53
- b) FADD
- c) MLKL
- d) GPX4

48. What is a typical hallmark of autophagy?

- a) activation of caspase-8
- b) formation of an autophagosome
- c) release of IL-1 β and IL-18
- d) MLKL-mediated membrane permeabilization

49. Which regulated form of cell death is linked to the inflammasome and IL-1 β release?

- a) NETosis
- b) anoikis
- c) ferroptosis
- d) pyroptosis

50. What is the principal significance of anoikis?

- a) defense against intracellular pathogens
- b) prevention of colonization by cells outside their tissue of origin
- c) degradation of damaged proteins
- d) maintenance of homeostasis during development

51. Which death mechanism is typically accompanied by the accumulation of lipid peroxides and the failure of GPX4?

- a) ferroptosis
- b) necroptosis
- c) NETosis
- d) autophagy

52. What is the main difference between the extrinsic and intrinsic apoptotic pathways?

- a) extrinsic is triggered by death receptors; intrinsic by DNA damage or cellular stress
- b) extrinsic is induced by oxidative stress; intrinsic by receptor ligands
- c) extrinsic occurs in lysosomes; intrinsic in the nucleus
- d) extrinsic leads to necrosis; intrinsic to apoptosis

53. Which multiprotein complex forms in the intrinsic pathway after cytochrome c is released from mitochondria?

- a) the apoptosome
- b) the inflammasome
- c) the proteasome
- d) the ribosome

54. Which hallmark of necrosis distinguishes it from apoptosis?

- a) formation of apoptotic bodies
- b) DNA fragmentation
- c) membrane rupture with induction of inflammation
- d) activation of caspase-9

55. Which type of cell death is typically associated with the formation of extracellular traps (NETs)?

- a) NETosis
- b) pyroptosis
- c) anoikis
- d) ferroptosis

Answer Key to Model Questions

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|-----------|----------|----------|
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| 3. TTFT | 22. TTTF | 41. TTFF |
| 4. TFFF | 23. TFFT | 42. TTTF |
| 5. TFFF | 24. TFFT | 43. TTTF |
| 6. TTTT | 25. TTFF | 44. TTTF |
| 7. TFFT | 26. TTFT | 45. TTFT |
| 8. TFFF | 27. TFFF | 46. TFFF |
| 9. TFFT | 28. FTFF | 47. TFFF |
| 10. TFFF | 29. TFTF | 48. FTFF |
| 11. TTTT | 30. TFFT | 49. FFTT |
| 12. FT TT | 31. TFFF | 50. FTFF |
| 13. TFTF | 32. TFTT | 51. TFFF |
| 14. TFFF | 33. TTFT | 52. TFFF |
| 15. FFTF | 34. TTFT | 53. TFFF |
| 16. FTFT | 35. TTFT | 54. FFTF |
| 17. TFTF | 36. TTFT | 55. TFFF |
| 18. TTFF | 37. TTTF | |
| 19. FTTF | 38. TTTF | |

8 Nucleic acids

Genetic information is stored primarily in DNA, whereas in RNA viruses it is carried by RNA. The principal function of nucleic acids is the storage and transmission of genetic information, ensured by the mechanisms of replication, transcription, and translation.

During replication, DNA is copied with high fidelity to pass genetic information to daughter cells. Genetic information is not used directly in the cell; it is first transcribed into RNA transcripts. RNA is a molecule with a specific function and limited lifespan that serves as a template for the synthesis of particular proteins. The structure of a protein is determined by the order of nucleotides in DNA, which are arranged into triplets (codons). The specific arrangement of triplets is called the genetic code. It is universal, non-overlapping, triplet in nature, and encodes 20 proteinogenic amino acids. Gene mutations and defects in the genetic code can result in the production of aberrant proteins or a loss of protein function.

Composition of nucleic acids

The basic unit of DNA and RNA is the **nucleotide**, composed of a nitrogenous base, a pentose sugar, and a phosphate group. DNA contains the sugar deoxyribose, whereas RNA contains ribose. Nitrogenous bases are divided into **purines** (adenine, guanine) and **pyrimidines** (cytosine, thymine in DNA, uracil in RNA). Nucleotides are linked by **phosphodiester** bonds in the 3'–5' direction. Elongation of a new strand is catalyzed by DNA polymerase, which operates strictly in the **5'→3' direction**. A nucleoside is formed by joining a nitrogenous base to a pentose sugar (ribose or deoxyribose) via an N-glycosidic bond. Unlike a nucleotide, it lacks a phosphate group; upon phosphorylation, it becomes a nucleotide and can be incorporated into DNA or RNA. Purine nucleosides include adenosine and guanosine, whereas pyrimidine nucleosides include cytidine, thymidine, and uridine. **Chargaff's rule** states that in double-stranded DNA, the amount of purines equals the amount of pyrimidines. Adenine always pairs with thymine and guanine with cytosine; thus, $A = T$ and $G = C$, which ensures the stability of DNA structure and the fidelity of its replication.

Structure of DNA

The **primary structure** is the linear sequence of nucleotides. The **secondary structure**, described by Watson and Crick in 1953, is a double-stranded antiparallel helix (a right-handed double helix, B-DNA). The strands are stabilized by base complementarity: adenine pairs with thymine via two hydrogen bonds, and guanine with cytosine via three. The backbone consists of alternating phosphate and deoxyribose, while the bases are oriented toward the interior of the helix. The tertiary structure is characterized by the formation of supercoils (supercoiling).

Conformational types of DNA

B-DNA is the most prevalent form of DNA. It is right-handed, with a diameter of 2.37 nm, 10.5 base pairs (bp) per turn, and a helical rise of 3.4 nm per turn. **A-DNA** is also right-handed, arises under dehydrating conditions, has a diameter of 2.6 nm, 11 bp per turn, and a helical rise of 2.46 nm per turn. **Z-DNA** is left-handed, has 12 bp per turn, a rise of 4.56 nm per turn, and is important in the regulation of gene expression. DNA contains major and minor grooves (1.2 nm and 0.6 nm), which serve as binding sites for regulatory proteins.

Types of DNA

Nuclear DNA is organized into structural and regulatory segments that together constitute the complex genome of a eukaryotic cell. It contains coding regions, i.e., structural genes responsible for protein synthesis, as well as genes for rRNA and tRNA that enable protein biosynthesis. A large fraction consists of noncoding sequences, including promoter and regulatory regions, introns, pseudogenes, and various repetitive DNA segments.

Unique sequences constitute approximately 60% of the genome and include protein-coding genes that usually occur once or in a small number of copies. The rest of the genome comprises **repetitive sequences** that may be arranged in tandem or dispersed. **Moderately repetitive** sequences include, for example, multiple copies of genes for rRNA, tRNA, or histones, which exist in numerous copies to meet the high demand for transcriptional products. **Highly repetitive** segments consist mainly of mobile genetic elements and satellite DNA. This group includes **LINE** elements (long interspersed nuclear elements), which represent approximately 21% of the human genome, and **SINE** elements (short interspersed nuclear elements), of which the best known are **Alu** sequences, present at roughly 13%. **Satellite** DNA also represents a significant fraction. It is divided into **macrosatellites**, **minisatellites**, and **microsatellites** and is often localized in centromeric and telomeric regions of chromosomes.

Repetitive DNA segments have not only structural and evolutionary significance but also practical applications. Minisatellites and microsatellites (**VNTR** – variable number of tandem repeats, and **STR** – short tandem repeats) are utilized in forensic genetics and individual identification. In contrast, CpG islands play a crucial role in regulating gene expression and studying epigenetic changes. Thus, nuclear DNA serves not only as the carrier of genetic information but also as a source of variation that can be exploited in diagnostics, genetic research, and medicine.

Mitochondrial DNA (mtDNA) is a small circular double-stranded DNA molecule located in mitochondria and is inherited exclusively maternally, i.e., from the mother to the offspring. Its transmission does not involve recombination, which makes it possible to trace the maternal lineage in populations and to use mtDNA in evolutionary and anthropological studies.

Human mtDNA is approximately 16.6 kb and contains **37 genes**: 13 genes encode proteins of the respiratory chain (complexes I, III, IV, and V), 2 genes encode mitochondrial rRNAs, and twenty-two genes encode tRNAs required for mitochondrial translation. The genome is highly compact, lacks introns, and contains only minimal noncoding regions. The most prominent is the so-called **D-loop** (displacement loop), which functions as a regulatory region for replication and transcription. Notably, mitochondria use a modified genetic code, for example, the codon UGA, which is a stop signal in nuclear DNA, encodes tryptophan in mitochondria.

Although mtDNA encodes some of its own proteins, most proteins required for mitochondrial function are encoded by the nuclear genome and imported into mitochondria after cytoplasmic synthesis. This fact points to the symbiotic origin of mitochondria and supports the endosymbiotic theory, according to which mitochondria originated from formerly free-living prokaryotic organisms. Additional features corroborate this origin, including the presence of 70S ribosomes in mitochondria and initiation of protein synthesis with formylmethionine, both typical of bacteria.

Mutations in mitochondrial DNA lead to defects in energy metabolism because they affect proteins of the respiratory chain and oxidative phosphorylation. The consequences are so-called mitochondrial diseases, which often affect tissues with high energy demands, such as the muscles and nervous system. Well-known clinical abnormalities include Kearns-Sayre syndrome (ocular myopathy with retinopathy and cardiomyopathy), Pearson syndrome (a disorder affecting hematopoiesis and the pancreas), and various mitochondrial disorders that cause muscle weakness, encephalomyopathy, deafness, or organ failure.

Plasmids are extrachromosomal genetic elements found primarily in bacterial cells alongside the nucleoid. They consist of double-stranded circular DNA molecules usually 2 to 15 kb in length (though size can vary significantly). A characteristic feature of plasmids is their ability to replicate independently of the bacterial chromosome. They often carry genes that enable conjugation (transfer of genetic material from one bacterium to another). Each plasmid contains its own origin of replication (ORI) and genes necessary for its reproduction.

In addition, plasmids may contain genes that provide an advantage to the host cell, such as antibiotic resistance or genes for specific enzymes, like hyaluronidase. Engineered plasmids frequently include restriction sites and selection markers, which is why they are used as vectors of genetic information in molecular biology. Plasmids capable of replicating in multiple host cells are called “shuttle” vectors and allow gene expression even in organisms in which the genes do not naturally occur.

RNA structure

The **primary structure** of RNA is the linear sequence of ribonucleotides composed of ribose, a phosphate group, and one of four bases – adenine, guanine, cytosine, and uracil (which replaces thymine found in DNA). The **secondary** structure of RNA forms through intramolecular hydrogen bonds between complementary bases within a single strand, resulting in the formation of loops. The **tertiary** structure of RNA is the spatial arrangement of secondary

motifs into more complex 3D conformations. It includes interactions between distant parts of the molecule and is stabilized by non-conventional bonds, such as Hoogsteen base pairing or interactions with metal ions. Thanks to its tertiary structure, RNA can adopt complex shapes that are crucial for its catalytic and regulatory functions.

Types of RNA

Messenger RNA (mRNA) is produced by the transcription of structural genes. In eukaryotes, the precursor form (hnRNA) is processed into mature mRNA through modifications such as the addition of a 5' cap and a poly(A) tail, which protect the molecule from degradation and enable efficient translation. The role of mRNA is to carry genetic information from the nucleus to the cytoplasm, where it serves as a template for protein synthesis on ribosomes. It carries the nucleotide sequence that is translated into the primary structure of proteins.

Ribosomal RNA (rRNA), together with proteins, forms ribosomes and constitutes up to 80% of cellular RNA. In eukaryotes, ribosomes are composed of the 40S and 60S subunits, which contain several types of rRNA (18S, 5.8S, 28S, and 5S). rRNA is synthesized in the nucleolus, where the precursor 45S rRNA undergoes processing and modifications. The resulting molecules associate with proteins to form functional ribosomes, which are then transported to the cytoplasm.

Transfer RNA (tRNA) mediates translation by carrying a specific amino acid to the ribosome and pairing its anticodon with the codon on mRNA. Each tRNA consists of approximately 74–95 nucleotides, contains modified bases, and has specific arms that form a cloverleaf structure. In three-dimensional space, it adopts an L-shape, allowing for the precise attachment of amino acids. At the 3' end, the CCA sequence serves as the site for amino acid binding. In the human genome, several hundred tRNA genes exist, organized into transcriptional units.

In addition to the main types, there are also non-coding RNAs. **Long non-coding RNAs** (lncRNAs) include, for example, XIST, which is involved in X-chromosome inactivation.

Circular RNA (circRNA) is a type of **non-coding RNA** generated through a process called back-splicing, in which the ends of the molecule join to form a closed loop. This structure increases its stability against degradation. CircRNAs often act as “sponges” for miRNAs, thereby indirectly regulating gene expression; they can also interact with proteins or influence transcription. They are characterized by tissue specificity, and altered expression is associated with various diseases.

Short non-coding RNAs (snRNA, snoRNA) participate in RNA processing and modification. Regulatory RNAs, including microRNA (miRNA), small interfering RNA (siRNA), and piRNA, regulate gene expression through RNA interference (RNAi). The most important are miRNAs, which bind complementary sequences on mRNAs and thereby block their translation. This mechanism regulates more than one-third of human genes. siRNA is double-stranded RNA

that induces post-transcriptional gene silencing, while piRNA protects the genome of germ cells from transposons. RNA interference is a crucial regulatory mechanism that can be leveraged in therapeutic strategies, but it is also linked to the development of certain diseases. Examples of different RNA types and their basic characteristics are given in the following table.

RNA type	Length / Size	Structure	Main Function	Localization
mRNA	variable	single-stranded	carries genetic information from DNA to the ribosome; serves as a template	nucleus and cytoplasm
tRNA	74–95 nt	single-stranded, cloverleaf secondary structure, L-shaped in 3D	delivers amino acids to the ribosome; decodes mRNA (anticodon)	cytoplasm
rRNA	5S, 5.8S, 18S, 28S (eukaryotes)	single-stranded, forms complex double-stranded and tertiary structures	component of ribosomes; catalyzes peptide bond formation	nucleolus and cytoplasm
snRNA	~150 nt	single-stranded	splicing of hnRNA, formation of the spliceosome	nucleus
snoRNA	60–300 nt	single-stranded	modification of rRNA (methylation, pseudouridylation)	nucleolus
miRNA	~22 nt	single-stranded	regulation of gene expression by binding to mRNA (translation block or degradation)	nucleus and cytoplasm
siRNA	20–25 nt	double-stranded, with overhanging nucleotides at the ends	post-transcriptional gene silencing, mRNA degradation	cytoplasm



Model questions

1. Which of the following applies to mitochondrial DNA?

- a) is paternally inherited
- b) is a large circular molecule
- c) contains 37 genes
- d) does not contain introns

2. Which of the following applies to A-DNA?

- a) is left-handed
- b) is right-handed
- c) occurs in conditions of excess water
- d) occurs in conditions of low salt

3. A nucleoside contains:

- a) sugar
- b) phosphate
- c) nitrogenous base
- d) glycosidic bond

4. DNA is found in:

- a) almost all cells
- b) the nucleus
- c) mitochondria
- d) the endoplasmic reticulum

5. Pyrimidine bases are:

- a) cytosine
- b) uracil
- c) thymine
- d) guanine

6. According to base-pairing rules, adenine pairs with:

- a) adenine
- b) guanine
- c) thymine
- d) uracil

7. Chargaff's rule states that:

- a) the amount of adenine = the amount of thymine
- b) the amount of guanine = the amount of cytosine
- c) the amount of purines equals the amount of pyrimidines
- d) the amount of purines equals the amount of pyrimines

8. Right-handed DNA conformations are:

- a) only Z
- b) only A
- c) only B
- d) A and B

9. In a polynucleotide chain, nucleotides are connected by:

- a) N-glycosidic bond
- b) 3,5-glycosidic bond
- c) 3,5-phosphate bond
- d) 3,5-phosphodiester bond

10. Which type of DNA is important for gene expression regulation?

- a) B-DNA
- b) Z-DNA
- c) A-DNA
- d) C-DNA

11. Which DNA segments make up the nuclear genome?

- a) coding genes (proteins, rRNA, tRNA)
- b) non-coding regions (pseudogenes, promoters, repetitive sequences)
- c) mitochondrial DNA
- d) histone proteins

12. Which genomic regions are highly repetitive sequences?

- a) LINE
- b) SINE-Alu
- c) satellite DNA
- d) histone genes

13. Which of the following applies to mitochondrial DNA (mtDNA)?

- a) contains introns
- b) has a slightly different genetic code
- c) encodes all mitochondrial proteins
- d) is inherited exclusively maternally

14. How many genes does human mtDNA contain?

- a) 13 protein-coding
- b) 22 for tRNA
- c) 2 for rRNA
- d) 46 in total

15. What does Chargaff's rule ensure?

- a) equal amounts of purines and pyrimidines
- b) stability of DNA structure
- c) accuracy of replication
- d) formation of supercoils

16. Which types of repetitive sequences are minisatellites and microsatellites?

- a) LINE
- b) SINE
- c) satellite DNA
- d) pseudogenes

17. Which molecule is the main carrier of genetic information in most organisms?

- a) DNA
- b) RNA
- c) proteins
- d) lipids

18. What does a nucleotide consist of?

- a) nitrogenous base
- b) pentose sugar
- c) phosphate group
- d) amino acid

19. Which sugar is found in DNA and which in RNA?

- a) deoxyribose in DNA
- b) ribose in RNA
- c) glucose in DNA
- d) fructose in RNA

20. Which bases are purines?

- a) adenine
- b) guanine
- c) cytosine
- d) thymine

21. Which bases are found in RNA?

- a) adenine
- b) guanine
- c) uracil
- d) thymine

22. How are nucleotides connected in a polynucleotide chain?

- a) peptide bond
- b) 3'–5' phosphodiester bond
- c) hydrogen bond
- d) disulfide bridge

23. How does a nucleoside differ from a nucleotide?

- a) nucleoside does not contain phosphate
- b) nucleoside always contains thymine
- c) nucleotide lacks ribose
- d) nucleotide contains phosphate

24. How many base pairs are there per turn in B-DNA?

- a) 10
- b) 10,5
- c) 11
- d) 12,5

25. Which base pair is connected by three hydrogen bonds?

- a) A – T
- b) G – C
- c) A – U
- d) T – U

26. What are the characteristics of Z-DNA?

- a) it is right-handed
- b) 12 bp per turn
- c) is important for gene expression regulation
- d) forms under dehydration

27. What is a characteristic feature of A-DNA?

- a) it is left-handed
- b) forms under dehydration
- c) 11 bp per turn
- d) found in mitochondria

28. Which sequences belong to non-coding regions of DNA?

- a) promoters
- b) introns
- c) rRNA
- d) exons

29. What is the proportion of LINE elements in the human genome?

- a) 5%
- b) 13%
- c) 21%
- d) 40%

30. What is the D-loop in mitochondrial DNA?

- a) rRNA-coding region
- b) tRNA-coding region
- c) regulatory region for replication and transcription
- d) codon for tryptophan

31. What is a typical feature of plasmids?

- a) are linear DNA molecules
- b) replicate independently of the main chromosome
- c) carry mitochondrial genes
- d) lack their own origin of replication

32. What genes can plasmids contain?

- a) antibiotic resistance
- b) specific enzymes
- c) hemoglobin gene
- d) histone gene

33. Which sugar is a part of a ribonucleotide?

- a) deoxyribose
- b) ribose
- c) glucose
- d) fructose

34. What allows the formation of RNA secondary structure?

- a) phosphodiester bonds
- b) hydrogen bonds between complementary bases
- c) disulfide bridges
- d) interactions with proteins

35. What stabilizes the tertiary structure of RNA?

- a) Hoogsteen pairings
- b) interactions with metal ions
- c) peptide bonds
- d) histone binding

36. Which rRNAs are present in eukaryotic ribosomes?

- a) 18S
- b) 28S
- c) 45S
- d) 5S

37. What is the shape of tRNA?

- a) helical spiral
- b) L-shape in 3D
- c) cloverleaf in 2D
- d) Y-shape

38. Which sequence is located at the 3' end of tRNA and serves for amino acid attachment?

- a) AUG
- b) CCA
- c) UGA
- d) GCU

39. Which type of RNA functions as a “sponge” for miRNA and thereby regulates gene expression?

- a) tRNA
- b) circRNA
- c) rRNA
- d) siRNA

40. What are the functions of miRNA?

- a) transport of amino acids
- b) blocking mRNA translation
- c) DNA repair
- d) activation of transcription

41. What is typical for siRNA?

- a) it is double-stranded
- b) causes post-transcriptional gene silencing
- c) activates transposons
- d) participates in ribosome formation

42. What is the main function of piRNA?

- a) regulation of more than one-third of genes
- b) protection of germ cell genomes from transposons
- c) mediating translation
- d) poly(A) tail formation

43. Approximately how many nucleotides are in tRNA?

- a) 20–40
- b) 50–60
- c) 74–95
- d) 120–150

44. Which small non-coding RNAs participate in RNA processing and modification?

- a) snRNA
- b) snoRNA
- c) miRNA
- d) rRNA

45. What is the main function of some circRNAs in gene expression regulation?

- a) serve as a template for histone synthesis
- b) act as “sponges” binding miRNAs
- c) degrade ribosomes
- d) activate translation in mitochondria

46. Which option about regulatory RNAs is correctly matched with their functions?

- a) miRNA – translation block, siRNA – mRNA degradation, piRNA – protection against transposons
- b) miRNA – protein synthesis, siRNA – splicing, piRNA – rRNA modification
- c) miRNA – catalysis of peptide bond, siRNA – transport of amino acids, piRNA – ribosome formation
- d) miRNA – elongation of translation, siRNA – activation of transcription, piRNA – protein degradation

47. RNA is:

- a) double-stranded
- b) single-stranded
- c) more stable than DNA
- d) not containing uracil

48. What is the typical spatial shape of transfer RNA (tRNA)?

- a) double-helix spiral
- b) letter L
- c) triple-helix spiral
- d) globular globulin

49. Which lncRNA is involved in X-chromosome inactivation?

- a) miRNA
- b) XIST
- c) snoRNA
- d) siRNA

50. Which types of RNA are involved in RNA processing and modification?

- a) snRNA and snoRNA
- b) mRNA and rRNA
- c) miRNA and siRNA
- d) circRNA and lncRNA

51. Which type of RNA is the most abundant in the cell?

- a) mRNA
- b) rRNA
- c) tRNA
- d) miRNA

52. What structure is formed during intramolecular base pairing within a single RNA strand?

- a) tertiary structure
- b) secondary structure
- c) primary structure
- d) quaternary structure

53. Which process is directly dependent on snRNA?

- a) splicing of hnRNA
- b) translation on ribosomes
- c) mRNA degradation
- d) formation of the poly(A) tail

54. Which of the following RNAs has the shortest length?

- a) mRNA
- b) tRNA
- c) miRNA
- d) snoRNA

55. Which option is correct about RNAs and their length?

- a) snoRNA – 60 to 300 nt
- b) snRNA – approximately 150 nt
- c) miRNA – 150 nt
- d) siRNA – 20 to 25 nt

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. FFTT | 20. TTFF | 39. FTFF |
| 2. FTFF | 21. TTTF | 40. FTFF |
| 3. TFTT | 22. FTFF | 41. TTFF |
| 4. TTTF | 23. TFFT | 42. FTFF |
| 5. TTTF | 24. FTFF | 43. FFTF |
| 6. FFTT | 25. FTFF | 44. TTFF |
| 7. TTTF | 26. FTTF | 45. FTFF |
| 8. FFFT | 27. FTTF | 46. TFFF |
| 9. FFFT | 28. TTFF | 47. FTFF |
| 10. FTFF | 29. FFTF | 48. FTFF |
| 11. TTFF | 30. FFTF | 49. FTFF |
| 12. TTTF | 31. FTFF | 50. TFFF |
| 13. FTFT | 32. TTFF | 51. FTFF |
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| 15. FTTF | 34. FTFF | 53. TFFF |
| 16. FFTF | 35. TTFF | 54. FFTF |
| 17. TFFF | 36. TTFT | 55. TTFT |
| 18. TTTF | 37. FTTF | |
| 19. TTFF | 38. FTFF | |

9 DNA replication

DNA replication is the process of copying genetic information that occurs during the S phase of the cell cycle. It is **semiconservative**, meaning that each newly formed double helix consists of one parental strand and one newly synthesized strand, thereby ensuring preservation of the original genetic information at every cell division. This mechanism was experimentally confirmed by Meselson and Stahl in 1958 and explains the high fidelity of replication and the genetic stability of the organism.

DNA replication in prokaryotic cells

DNA replication does not start at an arbitrary site along the chain, but at specific regions called origins of replication (**ORI**). The bacterium *E. coli* has a single circular DNA molecule and a single ORI of approximately 245 bp. **DnaA** proteins bind to this site and induce local unwinding of DNA. **DnaB** helicase then binds with the assistance of **DnaC**, which delivers it to the ORI. The **helicase** unwinds the double helix by disrupting hydrogen bonds between bases and creates a replication bubble that expands bidirectionally as two replication forks. The exposed single-stranded regions are stabilized by single-stranded binding proteins (**SSBs**), which prevent reannealing of the strands.

Primase, which has RNA polymerase activity, subsequently binds to the unwound DNA and, together with the helicase, forms the **primosome**. Primase synthesizes short RNA primers, which consist of 11 to 20 nucleotides. A single primer is sufficient on the leading strand, whereas multiple primers are generated repeatedly on the lagging strand, leading to the formation of **Okazaki fragments**. The actual strand elongation is carried out by **DNA polymerases**, which add new deoxyribonucleotides exclusively in the **5' → 3' direction**.

Five DNA polymerases have been identified in *E. coli* (the most important are DNA polymerases I–III). **DNA polymerase I** participates in removing RNA primers and replacing them with DNA; it also has 5' → 3' exonuclease activity and fills the gaps left after primer removal. **DNA polymerase II** functions in DNA repair and primarily plays a supportive role. **DNA polymerase III** is the primary replicative enzyme, working at a very rapid rate (250–1000 nucleotides per second). Its activity is supported by accessory proteins, such as the **β-clamp**, which increases processivity, and the **γ-complex**, which possesses ATPase activity.

Because DNA strands are antiparallel, they are synthesized in a different manner. The **leading** strand is replicated **continuously**, rapidly, and without interruption, whereas the **lagging** strand is replicated **discontinuously** in segments as Okazaki fragments, which in *E. coli* are approximately 2000 nucleotides long. Errors arising during replication are corrected by the proofreading activity of main DNA polymerases, which possess a 3'→5' exonuclease function.

After the removal of RNA primers by DNA polymerase I, the gaps are filled with DNA, and the individual segments are joined by **DNA ligase**, which forms phosphodiester bonds between fragments. Replication is thereby completed, yielding two identical DNA molecules, each containing one original and one newly synthesized strand.

DNA Replication in eukaryotic cells

In eukaryotic cells, DNA replication proceeds by mechanisms similar to those in prokaryotes but displays multiple specific features. To date, at least 15 types of DNA polymerases have been isolated from eukaryotic cells, with the most important ones having specialized functions. **DNA polymerase α** , together with primase, initiates DNA synthesis by producing RNA-DNA primers, including primers for Okazaki fragments. **DNA polymerase β** catalyzes short-patch DNA synthesis mainly during base excision repair. **DNA polymerase γ** is responsible for mitochondrial DNA replication and also contributes to mitochondrial DNA repair. **DNA polymerase δ** is the main lagging-strand polymerase and participates in Okazaki fragment elongation and maturation. **DNA polymerase ϵ** is generally considered the main leading-strand polymerase in eukaryotic nuclear DNA replication.

The eukaryotic replication apparatus also includes specific proteins, such as **PCNA** (proliferating cell nuclear antigen), which functions as the β -clamp, and **RFC** (replication factor C), which acts as a clamp loader analogous to the γ -complex in prokaryotes. Completion of the lagging strand differs from that in prokaryotic systems in that RNA primers are removed by a specialized exonuclease, **RNase H**. At the same time, the gaps are filled by DNA polymerase δ . Okazaki fragments are joined by DNA ligase.

A specific challenge for eukaryotic DNA is replicating the linear ends of chromosomes. The 3' end of the lagging strand cannot be fully replicated, leading to telomere shortening. **Telomeres** are tandem repeats rich in guanine; in humans, the sequence is TTAGGG. Their premature shortening is compensated by **telomerase**, a **specialized RNA-dependent DNA polymerase** that carries its own RNA template and adds short DNA repeats to the 3' end of the parental strand, thereby enabling DNA polymerase δ to complete synthesis of the lagging strand.

DNA replication in eukaryotes is roughly ten times slower than in prokaryotes, with polymerase progressing at about 50 nucleotides per second. This difference is compensated for by the formation of numerous replication origins in eukaryotic DNA, enabling parallel synthesis at thousands of sites. Okazaki fragments are also shorter, while prokaryotic fragments are 1,000-2,000 nucleotides long, and eukaryotic fragments are approximately 150 nucleotides long. Immediately after replication, chromatin structure is restored: nucleosomes disassemble during replication and are rapidly reassembled from newly synthesized histones, with each new nucleosome containing a combination of old and new histone subunits.

Differences in prokaryotic and eukaryotic replication are summarized in the following table.

Characteristics	Prokaryotes	Eukaryotes
DNA shape	circular, one molecule	linear, multiple chromosomes
ORI number	1 (oriC, ~245 bp)	thousands of ORI on each chromosome
Initiator complex	DnaA protein (binding to oriC)	ORC (Origin Recognition Complex)
replication duration	~40 min (<i>E. coli</i>)	8-12 hours (human cell)
polymerases	DNA pol. I – Elimination of primers and repair; DNA polymerase. II – repair; DNA pol. III – main replication	DNA pol. α -primase (primer synthesis), ϵ (leading strand), δ (lagging strand/Okazaki fragments), β (repair), γ (mtDNA)
synthesis speed	~1000 nt/s	~50 nt/s
Okazaki fragments	1000–2000 nt	~150 nt
deletion of RNA primers	DNA pol I (5'→3' exonuclease activity)	RNase H + Flap endonuclease 1 (FEN1)
fragment ligation	DNA ligase	DNA ligase
regulatory proteins	DnaA, DnaB (helicase), DnaC, SSB, gyrase	ORC, PCNA (β -clamp), RFC, helicases, RPA (SSB equivalent)
telomeres	DNA is circular without telomeres	telomerase ensures the replication of telomeres
chromatin	without histones	DNA associated with histones, after replication, follows the synthesis of new nucleosomes



Model questions

1. What is the role of the β -clamp in DNA chain polymerization?

- a) to bind DNA polymerase I to the DNA strand
- b) to bind RNA polymerase to the DNA strand
- c) to bind DNA polymerase III to the DNA strand
- d) to bind the parental and daughter DNA strands together

2. What is true about DNA polymerase I?

- a) participates in completing the leading strand
- b) participates in completing the lagging strand
- c) also has exonuclease activity
- d) is important in transcription

3. What is true about DNA polymerase II?

- a) consists of two polypeptide chains
- b) participates in DNA repair
- c) also has exonuclease activity
- d) is found in eukaryotes

4. What type of replication does DNA have?

- a) conservative
- b) semiconservative
- c) discontinuous
- d) radial

5. What is the function of SSB proteins?

- a) elongate the DNA chain
- b) stabilize single-stranded DNA
- c) unwind double-stranded DNA
- d) form Okazaki fragments

6. Which DNA polymerases have 3'→5' exonuclease activity?

- a) DNA polymerase I
- b) DNA polymerase II
- c) DNA polymerase III
- d) primase

7. Which processes occur on the lagging strand during replication?

- a) continuous synthesis
- b) discontinuous synthesis
- c) formation of Okazaki fragments
- d) formation of the β -clamp

8. Which DNA polymerases are responsible for DNA repair?

- a) DNA polymerase I
- b) DNA polymerase II
- c) DNA polymerase III
- d) primase

9. What is the main function of primase during replication?

- a) synthesizes short DNA primers
- b) synthesizes short RNA primers
- c) removes Okazaki fragments
- d) joins DNA fragments with ligase

10. Which DNA polymerase is the main replicative polymerase in *E. coli*?

- a) DNA polymerase I
- b) DNA polymerase II
- c) DNA polymerase III
- d) DNA ligase

11. Which statements are true about DNA replication?

- a) DNA polymerase adds nucleotides to both 5' and 3' ends of the growing RNA strand
- b) promoters are sequences signaling the start of transcription and are necessary for replication
- c) the DNA template strand is read in the 3'→5' direction
- d) both DNA strands serve simultaneously as templates

12. Double-stranded DNA replication occurs:

- a) semi-permeably
- b) semiconservatively
- c) conservatively
- d) via the Okazaki method

13. Phases of replication are:

- a) initiation
- b) catalysis
- c) termination
- d) elongation

14. DNA polymerases catalyze polymerization in the direction:

- a) bidirectional ($5' \rightarrow 3'$ and $3' \rightarrow 5'$)
- b) only $3' \rightarrow 5'$
- c) sometimes $5' \rightarrow 3'$
- d) only $5' \rightarrow 3'$

15. DNA ligase catalyzes the formation of:

- a) RNA primer
- b) phosphodiester bond between $5'$ and $3'$ ends of a polynucleotide chain
- c) phosphotriester bond between $5'$ and $3'$ ends of a polynucleotide chain
- d) formation of a complementary strand

16. Okazaki fragment synthesis occurs:

- a) in the direction of the replication fork
- b) against the direction of the replication fork
- c) discontinuously
- d) continuously

17. Primase is an enzyme that catalyzes:

- a) unwinding of complementary strands
- b) formation of bonds between DNA fragments
- c) DNA primer synthesis
- d) RNA primer synthesis

18. Okazaki fragment synthesis requires its own:

- a) RNA primer with a free $3'$ end
- b) DNA primer with a free $5'$ end
- c) RNA primer with a free $5'$ end
- d) DNA primer with a free $3'$ end

19. Which DNA polymerase synthesizes the leading strand?

- a) δ
- b) ϵ
- c) β
- d) γ

20. What is the main role of DNA polymerase α ?

- a) DNA repair
- b) mitochondrial DNA synthesis
- c) initiation of DNA replication
- d) proofreading

21. Which eukaryotic DNA polymerase catalyzes short-patch DNA synthesis mainly during base excision repair?

- a) δ
- b) ϵ
- c) β
- d) γ

22. Which DNA polymerase ensures mitochondrial DNA synthesis?

- a) δ
- b) ϵ
- c) γ
- d) β

23. Which protein functions as the β -clamp in eukaryotes?

- a) RFC
- b) PCNA
- c) RPA
- d) DNA ligase

24. Which protein in eukaryotes functions like the γ -complex?

- a) PCNA
- b) RFC
- c) DNA pol δ
- d) RNase H

25. Which enzyme removes RNA primers in eukaryotes?

- a) DNA pol I
- b) RNase H
- c) DNA pol δ
- d) DNA pol ϵ

26. Which enzyme joins DNA fragments in eukaryotes?

- a) DNA pol δ
- b) DNA ligase
- c) RFC
- d) PCNA

27. What causes the problem of incomplete replication at the 3' ends of DNA?

- a) circular genome
- b) linear DNA structure
- c) lack of histones
- d) excess introns

28. What is the human telomere sequence?

- a) TATAAT
- b) TTAGGG
- c) GACACA
- d) ATCGAT

29. DNA replication occurs:

- a) in the S-phase of the cell cycle
- b) in the G1-phase
- c) in the M-phase
- d) in the S-phase and is semiconservative

30. The origin of replication in *E. coli*:

- a) is called ORI
- b) is approximately 245 bp in size
- c) is designated as ORC
- d) is present on multiple chromosomes

31. The role of SSB proteins is:

- a) stabilize single-stranded DNA
- b) prevent strand reannealing
- c) catalyze RNA primer synthesis
- d) join Okazaki fragments

32. Which statements are correct about DNA polymerase I?

- a) removes RNA primers
- b) replaces RNA nucleotides with DNA nucleotides
- c) is the main replicative enzyme
- d) works at 250–1000 nt/s

33. The lagging strand:

- a) is synthesized continuously
- b) contains Okazaki fragments
- c) fragments are about 2000 nt in *E. coli*
- d) does not require primers

34. Replication fidelity is ensured by:

- a) polymerases with 3'→5' exonuclease activity
- b) polymerases performing proofreading
- c) ligase correcting wrong bases
- d) SSB proteins controlling correct base pairing

35. DNA replication termination involves:

- a) removal of RNA primers by DNA polymerase I
- b) joining DNA fragments by ligase
- c) degradation of the leading strand
- d) helicase inhibition

36. Which DNA polymerases participate in genome DNA replication in eukaryotic cells?

- a) DNA polymerase δ
- b) DNA polymerase α
- c) DNA polymerase II
- d) DNA polymerase IV

37. What are the functions of eukaryotic DNA polymerases?

- a) α makes primers, δ is the main lagging-strand polymerase and matures Okazaki fragments
- b) γ replicates mtDNA, β participates in base excision repair
- c) ϵ replicates the lagging strand, δ replicates the leading strand
- d) α replicates mtDNA, γ makes primers

38. Which proteins in eukaryotic replication have similar roles to the β -clamp and γ -complex in prokaryotes?

- a) PCNA
- b) RFC
- c) DnaA
- d) SSB

39. Which enzyme removes RNA primers during replication in eukaryotic cells?

- a) RNase H
- b) DNA polymerase δ
- c) DNA polymerase I
- d) ligase

40. What causes telomere shortening during replication?

- a) incomplete replication of the 3' end of the lagging strand
- b) loss of histones during nucleosome assembly
- c) activity of topoisomerase II
- d) limited telomerase activity

41. What are the characteristics of human telomeres?

- a) contain tandem repeats of TTAGGG
- b) are rich in guanine
- c) encode replication proteins
- d) exist only in mitochondria

42. Which statements are correct about DNA replication speed?

- a) is slower in eukaryotic cells than in prokaryotes
- b) eukaryotic polymerases add ~50 nt/s
- c) in prokaryotes, it is ~10 nt/s
- d) eukaryotes have only one ORI

43. Which statements are correct about Okazaki fragments?

- a) in eukaryotic cells, they are ~150 nucleotides long
- b) in prokaryotes, they are 1000–2000 nucleotides long
- c) formed continuously on the leading strand
- d) joined by topoisomerase

44. Which enzyme extends telomeres?

- a) telomerase
- b) specific RNA-dependent DNA polymerase with its own RNA template
- c) DNA polymerase I
- d) RNase H

45. What happens to nucleosomes during DNA replication in eukaryotes?

- a) disassemble into individual histones
- b) are completely replaced with new histones only
- c) remain firmly attached and unchanged
- d) reassemble from new and old histones after replication

46. What are the main differences between DNA replication in prokaryotes and eukaryotes?

- a) prokaryotes usually have one ORI
- b) in prokaryotes, DNA is linear
- c) eukaryotes have thousands of ORIs
- d) eukaryotes do not have histones

47. What is common for DNA polymerases of both prokaryotes and eukaryotes?

- a) they catalyze synthesis only in the 5' → 3' direction
- b) the main replicative ones have proofreading activity (3' → 5' exonuclease)
- c) they do not need a primer
- d) they join fragments via a phosphodiester bond

48. Which statements apply to Okazaki fragments?

- a) they enable lagging strand synthesis
- b) they are formed on the leading strand
- c) they arise only during mitochondrial DNA replication
- d) in eukaryotes, they are shorter (~150 nt) than in prokaryotes (~1000–2000 nt)

49. Which enzymes or mechanisms remove RNA primers?

- a) DNA polymerase I in prokaryotes
- b) RNase H and FEN1 in eukaryotes
- c) DNA polymerase γ
- d) DNA ligase

50. Which proteins ensure high processivity of DNA polymerase during replication?

- a) DnaA protein
- b) SSB proteins
- c) PCNA in eukaryotes
- d) β -clamp in prokaryotes

51. Which evidence supports the semiconservative mechanism of DNA replication?

- a) Griffith's experiment with pneumococci
- b) Meselson-Stahl experiment
- c) Avery's experiment with DNA
- d) Chargaff's rules

52. What is the role of DNA polymerase I in bacteria?

- a) synthesizes the leading strand
- b) removes RNA primers
- c) joins Okazaki fragments
- d) has 5' $\rightarrow$ 3' exonuclease activity

53. Which statements about eukaryotic DNA replication are correct?

- a) replication starts from a single ORI per chromosome
- b) Okazaki fragments are shorter than in prokaryotes
- c) RNA primers are removed by RNase H
- d) polymerase III is the main replicative enzyme

54. What is true about telomeres and telomerase?

- a) telomeres are present only in prokaryotes
- b) telomerase contains its own RNA template
- c) telomeres elongate spontaneously during each replication
- d) telomerase elongates the 3' end of the parental strand

55. What are the differences in speed and fragments during DNA replication?

- a) in eukaryotes the speed is ~ 50 nucleotides/s
- b) in prokaryotes the speed is slower (~ 10 nt/s)
- c) Okazaki fragments in prokaryotes are 1000–2000 nt
- d) Okazaki fragments in eukaryotes are longer than in prokaryotes

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. FFTF | 20. FFTF | 39. TFFF |
| 2. FTTF | 21. FFTF | 40. TFFT |
| 3. FTTF | 22. FFTF | 41. TTFF |
| 4. FTFF | 23. FTFF | 42. TTFF |
| 5. FTFF | 24. FTFF | 43. TTFF |
| 6. TTTF | 25. FTFF | 44. TTFF |
| 7. FTTF | 26. FTFF | 45. TFFT |
| 8. TTFF | 27. FTFF | 46. TFTF |
| 9. FTFF | 28. FTFF | 47. TTFF |
| 10. FFTF | 29. TFFT | 48. TFFT |
| 11. FFTT | 30. TTFF | 49. TTFF |
| 12. FTFF | 31. TTFF | 50. FFTT |
| 13. TFTT | 32. TTFF | 51. FTFF |
| 14. FFFT | 33. FTTF | 52. FTFT |
| 15. FTFF | 34. TTFF | 53. FTTF |
| 16. FTTF | 35. TTFF | 54. FTFT |
| 17. FFFT | 36. TTFF | 55. TFTF |
| 18. TFFF | 37. TTFF | |
| 19. FTFF | 38. TTFF | |

10 Protein synthesis

Structure and types of genes

A **gene** is the basic functional unit of genetic information. It is a **complete nucleic acid sequence** (DNA or RNA) that **carries the information necessary for the synthesis of a particular product**, either a functional RNA or a protein. Based on the type of genetic information, genes are divided into three main groups: structural genes, regulatory genes, and RNA-coding genes.

A **structural gene** is a segment of DNA (or RNA in RNA viruses) that encodes the primary structure of a **protein**. Gene size is given in base pairs (bp). A structural gene consists of a regulatory and a coding region. The **regulatory** region, called the **promoter**, contains specific sequences (“boxes”) that serve as binding sites for regulatory proteins (transcription factors). Proper assembly of these proteins enables the initiation of transcription (mRNA synthesis) by RNA polymerase.

A **prokaryotic promoter** contains two key regions. The first is the **–35 element**, located approximately at position –35 and usually containing the sequence 5'–TTGACA–3'. The second is the **–10 element**, also known as the **Pribnow box**, located at approximately position –10 and usually containing the sequence 5'–TATAAT–3'. These regions enable the recognition and binding of RNA polymerase.

Eukaryotic promoters are more complex, including several regulatory elements. The core element is the **TATA box (Hogness box)** located approximately at –34 to –26 with the sequence 5'–TATAAAA–3', which binds the transcription factor **TFIID**. Another important element is the **CAAT box**, located at –75 to –80, with the sequence 5'–GGCCAATCT–3', which binds NF1 to enhance promoter activity. Additionally, the **GC box**, located approximately at –90 with the sequence 5'–GGGCGG–3', binds SP1 and further enhances promoter activity.

In the **coding** region of eukaryotic genes, **exons** (coding segments) alternate with **introns** (non-coding segments). The **transcribed region** begins with the 5' untranslated region (5' UTR), which contains sequences important for translation initiation and recruitment of the mRNA to the small ribosomal subunit. The **coding sequence** begins at the start codon. The first exon begins with the start triplet ATG, followed by an intron, then the second exon, and so on; the last exon ends with a stop codon (TAA, TAG, or TGA). Transcription ends with the 3' UTR, followed by the sequence that activates poly(A) polymerase, which generates the poly(A) tail.

A **regulatory gene** is a DNA segment that encodes proteins functioning as **inducers** or **repressors** of other genes' expression. These genes regulate the activity of structural genes, enabling the cell to adapt to changing conditions. **RNA-coding** genes encode the primary

structure of ribosomal RNA (rRNA), transfer RNA (tRNA), and other small RNA molecules that perform specific cellular functions in addition to protein synthesis.

The **genetic code** is the method by which hereditary information is recorded. Its basic informational unit is the nucleotide sequence. The shortest meaningful unit for protein synthesis is the **triplet (codon)**, a set of three consecutive nucleotides in mRNA that specifies a particular amino acid in the polypeptide chain. The genetic code uses four bases to encode more than 20 amino acids, including a special 21st amino acid, selenocysteine. A combination of three bases provides $4^3 = 64$ possibilities, sufficient to encode all amino acids.

Codons in mRNA are read in the 5'→3' direction from the **start codon**. There are 64 codons in total: 61 encode amino acids, with AUG encoding methionine and usually signaling the start of translation in eukaryotes. UAA, UAG, and UGA are **stop codons** that do not encode an amino acid but signal translation termination.

The cell possesses a translational apparatus capable of reading the genetic code via tRNA and ribosomes. A gene is a complete unit of information that determines the primary structure of a single protein or an RNA product. The standard genetic code is universal for most organisms and consists of triplets that encode amino acids.

The **central dogma** of molecular biology explains the fundamental flow of genetic information in a cell—from DNA, through transcription into RNA, and then translation into protein. This principle was formulated by Francis Crick in 1958 and remains the foundation of molecular genetics. Later, exceptions to this principle were discovered. In 1970, Temin and Baltimore discovered the enzyme reverse transcriptase in retroviruses, which allows the flow of information from RNA back to DNA. Another specific deviation involves **prions** (pathogenic proteins), which do not contain nucleic acids but can change the conformation of normal PrP protein into the abnormal PrP^{Sc} form.

Transcription

During transcription, an mRNA molecule is synthesized according to the nucleotide sequence of DNA, which then moves from the nucleus to the ribosomes in the cytoplasm. In this process, only a specific segment of one of the two DNA strands is transcribed, either a single gene or a set of genes arranged in sequence. The transcription of DNA into RNA is catalyzed by the enzyme RNA polymerase, which adds nucleotides in the 5'→3' direction according to the principle of complementarity.

In **prokaryotes**, mRNA is produced **directly**, whereas in **eukaryotes**, a primary transcript (**precursor**) is synthesized and subsequently processed into functional RNA. The segment of DNA transcribed into the primary transcript is referred to as the transcription unit. In prokaryotes, a transcription unit may contain one or more structural genes, meaning that a single mRNA carries information for multiple polypeptide chains and is referred to as **polycistronic**.

Eukaryotic mRNAs are **monocistronic**, meaning they contain information for the synthesis of only one protein. The prokaryotic transcription unit is characterized by a leader sequence downstream of the promoter that includes the **Shine-Dalgarno sequence** (5'–AGGA–3'). This sequence enables the binding of mRNA to the 16S rRNA within the 30S ribosomal subunit, leading to the formation of the complete mRNA-ribosome complex. In prokaryotic cells, a single RNA polymerase enzyme synthesizes all types of RNA.

Transcription begins with **initiation**, when RNA polymerase binds the promoter via the **sigma (σ) factor** and locally unwinds the DNA to form a transcription bubble. The sigma factor ensures promoter-specific binding. During **elongation**, after promoter escape and sigma release, elongation factors such as **NusA** may associate with RNA polymerase. The enzyme moves along the DNA template, maintains the transcription bubble, and synthesizes RNA at approximately 60 nucleotides per second, while DNA renatures behind it. **Termination** occurs by **rho-independent or rho-dependent mechanisms**. In rho-independent termination, a GC-rich inverted repeat forms an RNA stem-loop followed by a **U-rich** tract; weak A-U pairing destabilizes the RNA-DNA hybrid and promotes transcript release. NusA can facilitate pausing and hairpin-mediated termination. In rho-dependent termination, rho (**ρ**) binds the nascent RNA and promotes transcript release. In prokaryotes, transcription is often coupled to translation, mRNA is usually short-lived, and ribosomes can bind while it is still being synthesized.

In **eukaryotic** cells, three types of RNA polymerases are responsible for catalyzing the synthesis of RNA. **RNA polymerase I** is located in the nucleolus and transcribes rRNA genes, producing a precursor rRNA (pre-rRNA) that is post-transcriptionally processed into 5.8S, 18S, and 28S rRNA. RNA polymerase II catalyzes the synthesis of mRNA by transcribing structural genes; its primary product is heterogeneous nuclear RNA (hnRNA, also known as pre-mRNA), which is subsequently processed into functional mRNA. It also synthesizes small nuclear RNAs and miRNAs. RNA polymerase III transcribes tRNA genes (pre-tRNA) and 5S rRNA, as well as certain small nuclear and cytoplasmic RNAs.

Transcription in eukaryotes requires accessory proteins, known as transcription factors, which ensure the recognition of promoter sequences, the recruitment of RNA polymerase II, local DNA unwinding, and the activation of the polymerase by phosphorylation. The intensity of transcription is further regulated by enhancers and silencers. **Initiation** proceeds via binding of a regulatory protein to the major groove of DNA upstream of the gene, disassembly of nucleosomes, and local linearization of DNA. A **helicase** separates the DNA strands, and transcription factors successively bind to the main promoter elements (TATA, CAAT, GC). The **TATA box** is crucial, as it binds the **TFIID protein** (TBP), forming the pre-initiation complex. RNA polymerase II and additional factors then associate, and **TFIIH** activates the polymerase by phosphorylation. Transcription begins at the 5' end of the transcribed region (producing the 5' UTR) and proceeds past the last exon into the 3' UTR. Termination of transcription of a structural gene is signaled by the polyadenylation signal AATAAA, which is transcribed as AAUAAA; at this site, a protein endonuclease complex cleaves the hnRNA, allowing its release from the transcriptional apparatus.

The difference between prokaryotic and eukaryotic transcription is presented in the table.

Phase	Prokaryotes	Eukaryotes
Type of RNA polymerase	one type of RNA polymerase (synthesizes all types of RNA)	RNA pol I → rRNA (besides 5S), RNA pol II → mRNA (hnRNA), snRNA, RNA pol III → tRNA, 5S rRNA, small RNA
Primary product	functional mRNA	primary transcript (hnRNA, pre-mRNA), requires modification (cap, splicing, polyA)
Transcriptional unit	1 or more genes (polycistronic mRNA)	usually monocistronic (1 gene → 1 mRNA)
Specific sequences	Shine-Dalgarno sequence (mRNA + 16S rRNA)	promoter boxes: TATA, CAAT, GC; termination AAUAAA (polyadenylation)
Initiation	RNA pol binds to the promoter via σ -factor → transcriptional bubble	requires transcriptional factors (TFIID, TBP, TFIIH), unwinds by DNA helicase, formation of the preinitiation complex
Elongation	speed ~60 nt/s; after release of σ -factor, NusA protein binds	RNA polymerase II phosphorylated by TFIIH; synthesis proceeds through exons and introns up to the 3' UTR
Termination	Rho-independent: polyU sequence (destabilization of A-U bonds), Rho-dependent: requires the ρ -factor	signaled by the polyadenylation signal (AAUAAA); the endonuclease complex cleaves hnRNA
Link between transcription and translation	tightly coupled: ribosomes bind to mRNA already during its synthesis	separated in space and time: transcription in the nucleus, translation in the cytoplasm
Lifespan of mRNA	very short (seconds to minutes)	relatively longer (minutes to hours)
Regulation	promoter + σ -factors, operons	transcription factors, enhancers, silencers, chromatin modification (nucleosome remodeling)

Post-transcriptional processing of pre-mRNA

The **primary transcript** must undergo a series of modifications known as **post-transcriptional modification** or **RNA maturation**. In prokaryotic cells, some mRNA molecules may become functional immediately after transcription, whereas rRNA and tRNA are processed from precursor molecules. In contrast, most eukaryotic RNAs undergo processing after transcription. The precursor of eukaryotic mRNA is pre-mRNA, historically called heterogeneous nuclear RNA (hnRNA), and is synthesized by RNA polymerase II. Its conversion into mature mRNA takes place in the nucleus and involves three main steps.

5' capping is one of the first steps. A guanine nucleotide is attached to the 5' end via an atypical 5'–5' triphosphate bond and is methylated at position 7. The cap protects RNA from exonucleases and promotes efficient translation initiation. The second major step is **splicing**. Introns are removed by spliceosomes, ribonucleoprotein complexes containing small nuclear

RNAs that recognize conserved intron boundaries, usually GU at the 5' end and AG at the 3' end. The intron is excised as a lariat structure, and the exons are joined by a phosphodiester bond. Another major step is **polyadenylation**. A poly(A) tail, usually consisting of approximately 100–250 adenylates, is added to the 3' end by poly(A) polymerase after cleavage of the transcript. This region protects the mRNA, facilitates nuclear export, and regulates stability and translation. The result is mature mRNA, which leaves the nucleus and associates with ribosomal subunits in the cytoplasm, where translation occurs.

Translation

Translation is the process that deciphers the genetic information in mRNA into the amino acid sequence of a protein. It occurs on ribosomes in the cytoplasm and comprises three main phases: initiation, elongation, and termination, followed by post-translational processing of the protein in the endoplasmic reticulum and Golgi apparatus.

During **initiation**, the first amino acid, methionine (formylmethionine in prokaryotes), binds directly to the P site of the ribosome, as only initiator tRNAs can occupy this site. mRNA binds to the small ribosomal subunit, along with initiation factors. The initiator tRNA then joins, and the large subunit attaches, forming a functional initiation complex ready for elongation.

During **elongation**, the second aminoacyl-tRNA enters the A site with correct codon-anticodon pairing ensured by elongation factor EF1 and GTP. If mismatched, the tRNA is replaced by the correct complementary one. Once paired, the amino acids in the P and A sites are joined by a peptide bond catalyzed by peptidyl transferase. The ribosome then shifts one codon forward in the 5'→3' direction, mediated by elongation factor EF2 and GTP. The aminoacyl-tRNA moves from the A to the P site, the tRNA in the P site shifts to the E site, and is released. This cycle repeats, with the ribosome always holding two active tRNAs (in the P and A sites) plus one exiting at the E site.

Termination occurs when the ribosome encounters a stop codon for which no tRNA exists. The final tRNA remains bound, and the release factor (RF), together with GTP, hydrolyzes the bond, freeing the completed polypeptide chain. The ribosome then dissociates from the mRNA and splits into subunits.

Multiple ribosomes can bind a single mRNA simultaneously, forming polyribosomes (**polysomes**) that enable concurrent synthesis of multiple copies of the same protein. The newly formed polypeptide is then directed to the endoplasmic reticulum and Golgi apparatus for post-translational modifications, where it acquires its final functional structure.

The following table summarizes translation.

Phase	Ribosome site	Function	Main processes
Initiation	P	mRNA, initiator tRNA (Met-tRNA / fMet-tRNA), small and large ribosomal subunits, initiation factors (IF / eIF)	Methionine binds to the P site, mRNA attaches to the small subunit, the large subunit joins, forming the functional initiation complex
Elongation	A, P, E	Aminoacyl-tRNA, elongation factors (EF1, EF2 + GTP), peptidyltransferase.	Aminoacyl-tRNA enters the A site, codon-anticodon pairing occurs; peptidyltransferase forms the peptide bond between amino acids; the ribosome translocates, tRNA moves from P to E site, and is released.
Termination	P	Stop codon is reached; release factors (RF) and GTP promote the release of the polypeptide chain.	The ribosome encounters a stop codon; the polypeptide is released; the ribosome dissociates into subunits and detaches from the mRNA
Polyribosome (polysome)	A, P, E	Multiple ribosomes simultaneously bind to a single mRNA	Allows the simultaneous synthesis of multiple copies of the same protein
Posttranslational modifications	-	endoplasmic reticulum, Golgi apparatus	The peptide undergoes processing and modifications to attain its final functional structure.

Post-translational modification of proteins

The initial segment of some newly synthesized polypeptides, called the **signal sequence (SS)** or **signal peptide**, directs the ribosome-nascent chain complex to the endoplasmic reticulum. This signal is recognized by the **signal recognition particle (SRP)**, which guides the ribosome to the ER membrane, where it binds to the **SRP receptor** and the **translocon**. The growing polypeptide then gradually enters the ER lumen, where the signal sequence is often cleaved and the protein may undergo early post-translational modifications. Proteins destined to become integral membrane proteins are inserted into the ER membrane. However, not all proteins enter the ER; many mature in the cytosol or are targeted to other compartments such as the nucleus, mitochondria, or peroxisomes. The primary polypeptide chain can be modified in various ways, including removal of the initial methionine or other amino acids, hydroxylation, phosphorylation, formation of disulfide bridges, folding into its tertiary structure, or proteolytic cleavage of a peptide segment.



Model questions

1. A structural eukaryotic gene contains:

- a) coding regions
- b) introns
- c) STOP codon
- d) START quadruplet

2. RNA polymerase I:

- a) transcribes tRNA genes
- b) is located in the nucleolus
- c) is present only in bacteria
- d) transcribes 5S rRNA genes

3. Transcription factor TFIID binds to:

- a) TATA box
- b) CAAT box
- c) GC box
- d) NusA protein

4. Prokaryotic mRNA is usually:

- a) megacistronic
- b) monocistronic
- c) acistronic
- d) polycistronic

5. Post-transcriptional modifications of RNA include:

- a) Shine-Dalgarno modification
- b) splicing of introns
- c) 5' cap
- d) 3' cap

6. Which of the following statements about RNA synthesis (transcription) are true?

- a) RNA polymerase adds nucleotides to the 5' → 3' growing DNA strand
- b) promoters are recognized sequences that signal the start of transcription
- c) the template DNA strand is read in the 3' → 5' direction
- d) both DNA strands serve as templates simultaneously

7. RNA polymerase II synthesizes:

- a) mRNA
- b) miRNA
- c) hnRNA
- d) tRNA

8. Amino acids can be coded by:

- a) always only one codon
- b) always only one triplet
- c) multiple triplets
- d) multiple codons

9. Modification of hnRNA includes:

- a) 5' end modification (cap)
- b) 3' end modification (cap)
- c) polyadenylation of the 3' end
- d) polyadenylation of the 5' end

10. Properties of the genetic code are:

- a) universal
- b) triplet
- c) degenerate
- d) semiconservative

11. The sequence of the start codon is:

- a) GUA
- b) UAG
- c) UAC
- d) AUG

12. Synthesis of the polypeptide chain in prokaryotic organisms begins with the amino acid:

- a) methionine
- b) N-formylmethionine
- c) arginine
- d) proline

13. Which of the following components are involved in the initiation of transcription?

- a) promoter region
- b) terminator sequence
- c) ribonucleoside triphosphates (rNTPs)
- d) transcription factors

14. What is meant by the term "genetic code"?

- a) the order of nucleotides determining the structure of a protein
- b) a chain of amino acids
- c) the type of RNA in a cell
- d) a sequence of repetitive DNA

15. Which enzyme elongates the ends of DNA strands during telomere replication?

- a) DNA pol ϵ
- b) telomerase
- c) DNA ligase
- d) PCNA

16. What happens to nucleosomes during DNA replication?

- a) nucleosomes remain unchanged during replication
- b) during replication, they disassemble and reassemble from old and new histones
- c) histones are completely removed and replaced only by new ones
- d) DNA replicates without histones

17. Which box is found in the prokaryotic promoter at position –10?

- a) GACA
- b) TATAAT (Pribnow box)
- c) CAAT
- d) GC

18. Which box is found in the eukaryotic promoter and binds TFIID?

- a) TATA (Hogness)
- b) CAAT
- c) GC
- d) Shine-Dalgarno

19. What alternates in the eukaryotic coding region?

- a) promoters and enhancers
- b) exons and introns
- c) ORF and 5' UTR
- d) GC and CAAT boxes

20. Which sequence signals polyadenylation of eukaryotic mRNA?

- a) AAUAAA
- b) AUG
- c) TTAGGG
- d) AATAAA

21. How many types of RNA polymerases do eukaryotes have?

- a) 1
- b) 2
- c) 3
- d) 5

22. Which RNA polymerase synthesizes mRNA in eukaryotes?

- a) RNA pol I
- b) RNA pol II
- c) RNA pol III
- d) RNA pol IV

23. Which sequence enables the binding of mRNA to 16S rRNA in prokaryotes?

- a) Shine-Dalgarno sequence
- b) TATA box
- c) PolyA signal
- d) GC box

24. Which DNA sequences regulate the intensity of transcription in eukaryotes?

- a) enhancers
- b) silencers
- c) σ factors
- d) nucleosomes

25. The mechanism of regulation of protein synthesis via RNA is called:

- a) interference
- b) interruption
- c) interaction
- d) intermediacy

26. The arms of tRNA are called:

- a) acceptor
- b) pseudouridine
- c) random
- d) codon

27. The protein synthesis machinery consists of:

- a) mRNA
- b) conformational ribosome
- c) amino acids
- d) tRNA

28. Translation takes place:

- a) in the nucleus
- b) in the nucleolus
- c) in the cytoplasm
- d) on ribosomes

29. The ribosome has the following binding sites:

- a) binding site for mRNA
- b) P site
- c) A site
- d) exit site

30. The subunits of eukaryotic ribosomes are:

- a) 50S and 30S
- b) 40S and 60S
- c) 60S and 30S
- d) 30S and 70S

31. Which sequence is present at the 3' end of tRNA?

- a) AUG
- b) UAA
- c) CCA
- d) TTAGGG

32. What is the function of adenine at the 3' end of tRNA?

- a) stabilization of the loop
- b) binding site for the amino acid
- c) binding to the ribosome
- d) binding to mRNA

33. Which bases belong to the minor bases of tRNA?

- a) inosine
- b) dihydrouracil
- c) thymine
- d) methionine

34. Which enzyme synthesizes precursor tRNA?

- a) RNA pol I
- b) RNA pol II
- c) RNA pol III
- d) DNA pol δ

35. Which enzyme removes introns from pre-tRNA?

- a) RNase H
- b) specific endonucleases
- c) exonuclease
- d) Pol δ

36. How many types of rRNA do prokaryotes have?

- a) 2
- b) 3
- c) 4
- d) 5

37. Which ribosomal subunits do eukaryotes have?

- a) 30S and 50S
- b) 40S and 60S
- c) 20S and 40S
- d) 18S and 28S

38. How many types of rRNA do eukaryotes have?

- a) 3
- b) 4
- c) 5
- d) 6

39. What does the 40S ribosomal subunit contain?

- a) 18S rRNA
- b) 28S rRNA
- c) 5S rRNA
- d) 5.8S rRNA

40. What does the 60S ribosomal subunit contain?

- a) 18S rRNA
- b) 28S rRNA
- c) 5.8S rRNA
- d) 5S rRNA

41. Where does rRNA synthesis occur?

- a) in the nucleus
- b) in the nucleolus
- c) in the cytoplasm
- d) in the endoplasmic reticulum

42. Which RNA polymerase transcribes 45S pre-rRNA?

- a) RNA pol I
- b) RNA pol II
- c) RNA pol III
- d) DNA pol γ

43. What is the main function of miRNA?

- a) catalysis of peptide bonds
- b) regulation of gene expression
- c) splicing of hnRNA
- d) transport of amino acids

44. What structure does siRNA have?

- a) single-stranded
- b) double-stranded
- c) circular
- d) cloverleaf

45. What is the main role of snoRNA?

- a) transfer of amino acids
- b) modification of rRNA
- c) regulation of mRNA
- d) translation

46. Which processes are mediated by snoRNA?

- a) methylation
- b) pseudouridylation
- c) phosphorylation
- d) polyadenylation

47. What is RNA interference (RNAi)?

- a) a splicing mechanism
- b) a mechanism of mRNA degradation or inhibition mediated by regulatory RNAs
- c) a protein synthesis mechanism
- d) a tRNA synthesis mechanism

48. Which enzyme catalyzes the formation of the peptide bond during elongation?

- a) peptidyl transferase
- b) DNA pol δ
- c) RNA pol II
- d) ligase

49. What are polyribosomes (polysomes)?

- a) chains of ribosomal proteins
- b) multiple ribosomes simultaneously on a single mRNA
- c) ribosomes attached to the ER
- d) groups of ribosomes without RNA

50. What is a gene?

- a) a segment of DNA or RNA carrying information for the synthesis of a protein or functional RNA
- b) a segment of DNA that always encodes only a protein
- c) the smallest unit of heredity is one nucleotide
- d) a complete DNA sequence that replicates

51. What types of genes do we distinguish according to their function?

- a) structural genes
- b) regulatory genes
- c) lipid-encoding genes
- d) polysaccharide-encoding genes

52. Which elements are present in a prokaryotic promoter?

- a) TTGACA box at position -35
- b) TATAAT (Pribnow) box at position -10
- c) CAAT box
- d) GC box

53. Which sequences are typical for a eukaryotic promoter?

- a) GACA box
- b) CAAT box
- c) TATA box (Hogness)
- d) Shine-Dalgarno sequence

54. What is found in the transcribed region of eukaryotic genes?

- a) only regulatory proteins
- b) introns
- c) only stop codons
- d) exons

55. What is a regulatory gene?

- a) a gene that encodes inducers or repressors affecting the expression of other genes
- b) a gene that is always transcribed into tRNA
- c) a gene without a function
- d) a gene that encodes poly(A) polymerase

56. How many codons are there and how many of them code for amino acids?

- a) 64 codons, 61 encode amino acids
- b) 64 codons, 3 are stop codons
- c) 20 codons, all encode amino acids
- d) 64 codons, only 20 are essential

57. The start codon:

- a) is UAA
- b) encodes methionine
- c) is found only in prokaryotes
- d) is AUG

58. What are prions?

- a) pathological proteins capable of changing the conformation of normal proteins
- b) particles containing only RNA
- c) infectious particles without nucleic acids
- d) retroviruses

59. What is transcription?

- a) synthesis of RNA from a DNA template
- b) synthesis of DNA from RNA
- c) protein synthesis on a ribosome
- d) repair of damaged DNA segments

60. What is the difference between mRNA in prokaryotes and eukaryotes?

- a) prokaryotic mRNA is often polycistronic
- b) eukaryotic mRNA is normocistronic
- c) prokaryotic mRNA always encodes only one protein
- d) eukaryotic mRNA is never modified

61. What mediates the initiation of transcription in prokaryotes?

- a) sigma factor
- b) NusA protein
- c) rho factor
- d) DNA ligase

62. What is characteristic of transcription elongation in prokaryotes?

- a) RNA polymerase proceeds at about 60 nt/s
- b) sigma factor remains bound
- c) NusA protein binds to RNA polymerase
- d) polymerase moves only along the leading DNA strand

63. What is the primary product of transcription of structural genes in eukaryotes?

- a) pre-mRNA
- b) mature mRNA
- c) tRNA
- d) hnRNA

64. What is a promoter?

- a) a regulatory region of a gene allowing RNA polymerase binding
- b) a DNA segment that always encodes a protein
- c) part of a ribosome
- d) a regulatory sequence containing boxes (e.g., TATA, CAAT)

65. Which codons are stop codons?

- a) UAA
- b) UAG
- c) UGG
- d) UGA

66. Which statements about exons and introns are correct?

- a) exons are retained segments of a gene
- b) introns are non-coding segments of a gene
- c) introns encode proteins
- d) exons are excised from the mature transcript

67. What is the difference between a structural and a regulatory gene?

- a) a structural gene encodes a protein
- b) a regulatory gene encodes a factor influencing the expression of other genes
- c) a regulatory gene always encodes tRNA
- d) a structural gene does not contain a promoter

68. What is the Shine-Dalgarno sequence?

- a) a region of prokaryotic mRNA that binds to 16S rRNA
- b) a region of the eukaryotic promoter
- c) the start codon AUG
- d) part of the poly(A) tail

69. Which statements are true about the start codon?

- a) is universally the sequence AUG
- b) encodes methionine
- c) is located in an intron
- d) is also a stop signal

70. What bases are read in the triplet codon?

- a) 2 purines and one pyrimidine
- b) three consecutive nucleotides
- c) 4 nucleotides in a row
- d) always only adenine and uracil

71. Introns are:

- a) non-coding segments of a gene
- b) removed by splicing
- c) codons for stop signals
- d) sequences that encode tRNA

72. Which amino acids are encoded by only one codon?

- a) methionine
- b) leucine
- c) tryptophan
- d) serine

73. What is found in the 3' UTR region of a gene?

- a) signals for polyadenylation
- b) regulatory sequences affecting mRNA stability
- c) start codon
- d) Shine-Dalgarno sequence

74. What is the GC box?

- a) a sequence of the eukaryotic promoter
- b) part of the poly(A) tail
- c) it binds the transcription factor SP1
- d) the site where the splicing begins

75. Which statements are correct about stop codons?

- a) there are three: UAA, UAG, UGA
- b) encode methionine
- c) do not specify any amino acid
- d) determine the beginning of translation

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. TTTF | 26. TTFF | 51. TTFF |
| 2. FTFF | 27. TFTT | 52. TTFF |
| 3. TFFF | 28. FFTT | 53. FTTF |
| 4. FFFT | 29. TTTT | 54. FTFT |
| 5. FTTF | 30. FTFF | 55. TFFF |
| 6. FTTF | 31. FFTF | 56. TTFF |
| 7. TTTF | 32. FTFF | 57. FTFT |
| 8. FFTT | 33. TTTF | 58. TFTF |
| 9. TFTF | 34. FFTF | 59. TFFF |
| 10. TTTF | 35. FTFF | 60. TFFF |
| 11. FFFT | 36. FTFF | 61. TFFF |
| 12. FTFF | 37. FTFF | 62. TFTF |
| 13. TFTT | 38. FTFF | 63. TTFT |
| 14. TFFF | 39. TFFF | 64. TTFT |
| 15. FTFF | 40. FTTF | 65. TTFT |
| 16. FTFF | 41. FTFF | 66. TTFF |
| 17. FTFF | 42. TFFF | 67. TTFF |
| 18. TFFF | 43. FTFF | 68. TFFF |
| 19. FTFF | 44. FTFF | 69. TTFF |
| 20. TFFF | 45. FTFF | 70. FTFF |
| 21. FFTF | 46. TTFF | 71. TTFF |
| 22. FTFF | 47. FTFF | 72. TFTF |
| 23. TFFF | 48. TFFF | 73. TTFF |
| 24. TTTF | 49. FTFF | 74. TFTF |
| 25. TFFF | 50. TFFF | 75. TFTF |

11 Methods in molecular genetics. Omics

The basis of every analysis is the proper **collection** and **processing** of biological material. DNA or RNA can be obtained from peripheral blood, buccal mucosa, chorionic villi, amniotic fluid, tissues, saliva, as well as from older samples fixed in formalin and embedded in paraffin. Forensic applications use swabs from the oral cavity, hairs with roots, teeth, or bones. In cases of infection, viruses and bacteria may serve as the source. Before isolation, the sample is homogenized mechanically, physically, or chemically using detergents. This is followed by centrifugation and fraction separation.

Isolation of nucleic acids removes proteins and polysaccharides while preserving the structure of DNA or RNA. After cell lysis, nucleases are inhibited. RNase is added during DNA isolation, and DNase is added during RNA isolation. Protein complexes are disrupted using organic solvents, DNA is precipitated with salts and alcohol, and subsequently dissolved in water. The concentration and quality of the isolated DNA are determined by several methods. In electrophoresis, DNA appears in the gel as a single thick band, indicating that the strands remain intact. Spectrophotometry measures the absorbance of DNA, providing information about its concentration. In fluorimetry, DNA is stained with a fluorescent dye that binds to the double helix, and the fluorescence intensity is directly proportional to the amount of DNA, allowing for a more precise estimation of concentration. However, a reliable quality control method for determining whether DNA is fragmented is provided only by electrophoresis.

Electrophoresis is among the most commonly used methods for separating nucleic acids and proteins based on size, charge, or binding affinity. DNA and RNA carry a negative charge due to their phosphate groups and therefore migrate toward the anode in an electric field. Separation does not occur directly in solution but on solid supports, most commonly in agarose or polyacrylamide gels. DNA samples are loaded into wells in the gel, along with a buffer solution that facilitates the conduction of current. Horizontal electrophoresis is typically used for DNA (in agarose), vertical electrophoresis for proteins (in polyacrylamide), and capillary electrophoresis for RNA analysis or sequencing.

The migration speed of molecules depends on their size, conformation, gel concentration, voltage, and buffer composition. Smaller fragments migrate faster than larger ones, circular DNA moves faster than linear DNA, and RNA migrates faster than DNA. The size of unknown fragments is determined by comparison with a DNA marker (ladder) containing fragments of known length.

DNA itself is not visible to the naked eye, therefore, it is visualized using fluorescent dyes. Traditionally, ethidium bromide has been used, which intercalates between DNA bases and fluoresces under UV light. Modern dyes such as SYBR® Green, GoldView®, or GelRed have lower toxicity and mutagenicity. The results are documented using UV transilluminators and photodocumentation systems.

Restriction endonucleases are bacterial enzymes that, within the restriction-modification system, cleave foreign DNA at specific palindromic sequences, while the host DNA is protected by methylation. According to the way a given restriction endonuclease cleaves double-stranded DNA and the type of ends it produces, three basic types are distinguished. Endonucleases can generate 5' overhangs, so-called sticky ends (e.g., *Sau3AI*, *ClaI*, *EcoRI*), 3' overhangs, also sticky ends (e.g., *HhaI*, *PstI*), or blunt ends (e.g., *AluI*, *SmaI*, *PvuII*).

DNA polymorphism refers to the presence of multiple variants of a specific DNA segment in a population, each occurring with significant frequency. It therefore represents genetic variability among individuals. For its detection, the Southern blot method, using labeled probes, was originally employed. Today, PCR, restriction enzymes, or sequencing are more commonly applied.

Single-nucleotide polymorphism (SNP) arises from the substitution of a single nucleotide or a base pair in DNA. If the change occurs within a restriction enzyme recognition site, the sequence will either be cleaved or not, this underlies the principle of RFLP (restriction fragment length polymorphism). The most common form of point polymorphism is the SNP, i.e., a change of a single nucleotide. SNPs occur approximately once every 300 nucleotides, predominantly in noncoding regions, but thousands are also found within genes. Functional SNPs can influence gene expression and are associated with multifactorial diseases.

Hypervariable regions of DNA are segments with varying numbers of short sequence motifs. When the repeats are arranged directly adjacent to each other, they are referred to as **tandem** repeats. The number of repeats differs among individuals, making these regions highly variable. Their analysis utilizes PCR and electrophoresis, which reveal differences in fragment size.

From a practical perspective, **point** polymorphisms (especially SNPs) are important for studying **gene function** and genetic predisposition to diseases. In contrast, hypervariable regions are widely used in **criminalistics** and **forensic** genetics for individual identification.

The **RFLP** method is based on the cleavage of genomic DNA by restriction endonucleases. These enzymes recognize specific short palindromic sequences in DNA and cut precisely at these sites. Since the corresponding recognition sites are present in large numbers throughout the genome, the result is an extensive collection of DNA fragments of varying lengths. The resulting double-stranded fragments are separated by size using electrophoresis in an agarose gel and are then denatured to generate single strands suitable for hybridization with specific probes. If a mutation (e.g., a nucleotide substitution) occurs within a palindromic site, the recognition sequence is lost and the corresponding fragment is not cleaved. Conversely, a mutation can create a new palindrome, thereby generating a new cleavage site. The result is the appearance of fragments of different lengths that reflect the presence or absence of specific polymorphisms. These fragment length differences among individuals constitute the essence of RFLP polymorphism.

Polymerase chain reaction (PCR) is a method that allows *in vitro* amplification of specific DNA segments of precisely defined length and location. It is among the most widely used molecular genetic techniques.

The **principle** of PCR lies in **mimicking DNA replication** under laboratory conditions, with synthesis restricted to a predetermined fragment bounded by oligonucleotide primers. Primers bind to complementary sequences flanking the target region, and their **3'-OH ends** permit synthesis of new strands by a thermostable DNA polymerase. The most commonly used enzyme is Taq polymerase, isolated from the bacterium *Thermus aquaticus*, which is able to withstand high temperatures.

DNA amplification occurs in a device called a thermal cycler, consisting of repeated steps: **denaturation** of double-stranded DNA at high temperatures, primer **annealing** at lower temperatures, and **elongation** of the new strand by Taq polymerase. Before the reaction begins, a reaction mixture is prepared containing template DNA, two primers (forward and reverse), dNTPs, Taq polymerase, MgCl₂ as a source of Mg²⁺ ions required for polymerase activity and reaction specificity, and sterile water. Each PCR cycle theoretically doubles the amount of target DNA, resulting in an exponential increase in the amount of product. After 25 to 35 cycles, approximately 10⁷- to 10¹⁰-fold amplification of the desired fragment can be achieved.

Quantitative PCR (real-time PCR) is a method that enables **precise quantification of DNA** by monitoring the formation of PCR products during each amplification cycle. Detection is based on fluorescence, generated either by dyes that bind specifically to double-stranded DNA or by hybridization probes containing a fluorescent reporter and a quencher. In an intact probe, fluorescence is quenched. During PCR, the 5' nuclease activity of DNA polymerase degrades the probe, separating the reporter from the quencher and generating a fluorescent signal. Fluorescence intensity increases proportionally with the amount of PCR product and is monitored after each cycle; the key parameter is the threshold cycle (Ct). The higher the Ct value, the smaller the initial amount of DNA.

Real-time PCR is used to measure gene expression (preceded by reverse transcription of RNA to cDNA), to determine gene copy number, in genotyping, and in microbiological diagnostics for rapid identification and quantification of pathogens without the need for culture. According to the approach, one distinguishes relative quantification, in which the amount of PCR product is compared with a reference standard, and absolute quantification, which determines the exact amount of DNA in copy numbers or mass units.

DNA sequencing is a set of methods that allows the determination of the **precise order of nucleotides** in DNA. Classic techniques include the Sanger method and the Maxam-Gilbert method.

The Sanger method utilizes dideoxynucleotides (ddNTPs), which terminate DNA synthesis upon incorporation. DNA polymerase, in the presence of primers and normal deoxyribonucleotides, synthesizes new strands, and the random incorporation of ddNTPs

produces fragments of varying lengths. These fragments are separated by electrophoresis, and their nucleotide sequences are read. Modern sequencers use fluorescently labeled ddNTPs, PCR to amplify target regions, and capillary electrophoresis, with a laser detecting colored signals and software generating the sequencing trace.

The Maxam-Gilbert method is based on the chemical modification of individual bases, followed by DNA cleavage, which produces fragments of different lengths. This method is now rarely used.

A major advancement is **next-generation sequencing** (NGS), which allows high-throughput processing of thousands to billions of DNA fragments simultaneously. On the Illumina platform, DNA is fragmented, the segments are attached to a sequencing chip, and DNA polymerase incorporates fluorescently labeled nucleotides one by one. Each type of nucleotide generates a distinct signal, which is detected by imaging devices. Bioinformatic processing then assembles the short reads into a complete sequence.

NGS is used for whole-genome sequencing, targeted DNA or RNA analysis, and epigenetic studies. Compared to the Sanger method, it is significantly faster, allows massive parallelization, and can process large amounts of data simultaneously. In contrast, classical Sanger sequencing is limited to a single DNA fragment.

Biochips represent a modern form of commercially available membranes on which probes designed to hybridize with the analyzed DNA are immobilized. While older membranes allowed the placement of tens of thousands of probes, today's biochips are about the size of a postage stamp. They can contain over 100,000 oligonucleotide probes with known sequences, enabling the parallel analysis of a large number of parameters, including all known polymorphisms and mutations of a single gene or multiple genes associated with polygenic diseases. The analyzed DNA or mRNA (converted into cDNA) is first fluorescently labeled and applied to the chip surface using a microrobotic system. Complementary hybridization occurs between the probes and the sample. Unbound sequences are then washed away, and the hybridization signal is detected based on fluorescence. For the detection of SNPs, comparison with a control sample labeled with a different color is often used, allowing precise discrimination between homozygous and heterozygous alleles. Biochips are used not only to compare DNA from healthy and diseased individuals but also for gene expression analysis via mRNA. A similar principle is applied in protein microarrays, which contain immobilized antibodies specific to selected proteins.

Omics

Modern molecular biology currently focuses not only on the study of individual genes or proteins but also on complex systems that determine the function of cells and organisms. **Genomics** examines the complete set of DNA of an organism, i.e., its genome, and allows the identification of genes, genetic variants, and mutations. In practice, next-generation sequencing

(NGS), a high-throughput technology that enables the rapid and accurate sequencing of the entire genome, is often employed. Genomics thus provides the foundation for understanding inheritance, genetic predisposition to diseases, and evolutionary relationships among organisms.

Transcriptomics focuses on all RNA molecules expressed in cells, collectively referred to as the transcriptome. Analysis of the transcriptome, particularly mRNA, enables the monitoring of gene expression levels and changes during development or in disease states. RNA sequencing is a key technique for transcriptomic studies, providing both quantitative and qualitative data on transcripts.

Proteomics examines the complete set of proteins produced by a cell, tissue, or organism at a given time and under specific conditions. Since proteins are the primary functional molecules of the cell, their analysis provides insight into regulatory mechanisms, signaling pathways, interactions, and post-translational modifications. Proteomics primarily uses mass spectrometry and two-dimensional gel electrophoresis and is crucial for biomarker research, personalized medicine, and the development of new therapeutic strategies.

Metabolomics focuses on small molecules and metabolites present in cells, tissues, or organisms. Analysis of metabolomes provides insight into cellular metabolic pathways, physiological state, and responses to external stimuli. Analytical methods such as mass spectrometry and nuclear magnetic resonance (NMR) are used to identify and quantify metabolites.

Viromics is a discipline focused on viral populations, their genetic diversity, interactions with host cells, and effects on the organism. Viromics also includes the study of the host-virus interactome, the network of interactions between viruses and the host.

By integrating genomics, transcriptomics, proteomics, and metabolomics, a **multi-omics** approach emerges that enables a comprehensive understanding of biological processes, disease states, and the mechanisms underlying their origin. The development of modern technologies has led to the emergence of additional specialized fields alongside the traditional disciplines mentioned above, providing an even more comprehensive picture of the functioning of biological systems.

Epigenomics focuses on chemical modifications of DNA and histones, such as methylation or acetylation, which do not change the DNA sequence itself but profoundly influence gene expression. It is key for research in cancer, aging, and environmental impacts.

Interactomics studies networks of interactions between proteins and other molecules, enabling an understanding of the dynamics of cellular processes and signaling pathways.

Lipidomics focuses on lipids and their roles in cell membranes, metabolism, and regulation. At the same time, glycomics examines the structure and function of carbohydrates and glycans, which significantly affect immune responses and pathological processes, including cancer.

Microbiomics analyzes the composition and function of microbial communities, particularly the human gut microbiome, and their impact on immunity, metabolism, and disease development.

Pharmacogenomics connects genetic knowledge with clinical practice by studying genetic factors that influence an individual's response to treatment, forming the basis of personalized medicine.

In recent years, **exosomics**, or extracellular vesiculomics, has emerged, which studies the contents of small vesicles released by cells. These vesicles carry nucleic acids, proteins, and lipids and represent a promising source of non-invasive biomarkers for diagnostic and prognostic applications.



Model questions

1. A restriction enzyme cuts DNA:

- a) specifically
- b) non-specifically
- c) at any site
- d) naturally in bacteria

2. The movement of DNA in a gel during electrophoresis depends mostly on:

- a) pH
- b) molecule size
- c) gravity
- d) gel concentration

3. Types of sequencing include:

- a) automated
- b) manual
- c) next-generation
- d) old-generation

4. After restriction enzyme digestion, the types of ends produced are:

- a) sticky in RNA
- b) blunt in DNA
- c) blunt in RNA
- d) sticky in DNA

5. What is necessary for a PCR reaction?

- a) Taq polymerase
- b) rRNA
- c) many DNA molecules
- d) dNTPs

6. Micropipettes:

- a) are used to handle microvolumes
- b) are not precise
- c) are used with tips
- d) can be adjustable

7. What is the main function of DNA sequencing?

- a) amplification of DNA fragments
- b) determination of DNA fragment size
- c) determination of nucleotide order in DNA
- d) measurement of gene expression

8. In paternity testing, restriction endonucleases allow:

- a) determination of the child's gender
- b) identification of DNA sequences specific to the biological father
- c) determination of blood type
- d) measurement of the child's height

9. Which type of sample is often used in forensics for paternity testing?

- a) blood serum
- b) buccal mucosa
- c) muscle tissue
- d) cerebrospinal fluid

10. Which archival material is mostly used in molecular diagnostics?

- a) cryopreserved cells
- b) formalin-fixed paraffin-embedded tissue
- c) raw tissue
- d) lyophilized DNA

11. What is the goal of tissue homogenization during DNA isolation?

- a) increase solution viscosity
- b) disrupt cells and release DNA
- c) activate nucleases
- d) prepare proteins

12. Which of the following belongs to physical homogenization methods?

- a) ultrasonication
- b) repeated freezing and thawing cycles
- c) use of detergents
- d) protease digestion

13. Which homogenization method is chemical?

- a) ultrasound
- b) hypotonic treatment
- c) detergents
- d) liquid nitrogen

14. What is the main role of centrifugation after homogenization?

- a) remove RNA
- b) remove PCR inhibitors
- c) separate solid particles
- d) dissolve DNA

15. Which enzymes are added during DNA isolation to remove RNA?

- a) DNase
- b) RNase
- c) protease
- d) lyase

16. Which organic solvents are used in DNA isolation?

- a) phenol
- b) chloroform
- c) urea
- d) acetone

17. DNA in solution is precipitated using:

- a) ethanol or isopropanol
- b) acetone
- c) sulfuric acid
- d) proteases

18. What characterizes good-quality DNA on agarose gel?

- a) diffuse bands
- b) a single thick band
- c) many short fragments
- d) fluorescence gradient

19. Why do nucleic acids have a negative charge?

- a) due to phosphate groups
- b) due to ribose
- c) due to hydrogen bonds
- d) due to nitrogenous bases

20. Which gel is used for DNA electrophoresis?

- a) polyacrylamide
- b) agarose
- c) cellulose
- d) polyurethane

21. What is the purpose of a DNA marker (ladder)?

- a) increase fluorescence intensity
- b) determine fragment size
- c) prevent DNA degradation
- d) improve migration

22. What is the most common form of point polymorphism?

- a) VNTR
- b) SNP
- c) STR
- d) CNV

23. What is the principle of Sanger sequencing?

- a) chemical base modification
- b) use of dideoxynucleotides
- c) use of proteases
- d) use of RNase

24. What is the principle of electrophoresis?

- a) migration of molecules in an electric field according to size and charge
- b) separation by density in a sucrose gradient
- c) transport of DNA through a membrane by phosphorylation
- d) DNA degradation by enzymes

25. Which type of gel is most commonly used to separate DNA?

- a) polyacrylamide
- b) agarose
- c) starch gel
- d) silica gel

26. What is an SNP (single-nucleotide polymorphism)?

- a) a point mutation that occurred in every cell
- b) a polymorphism in mitochondrial DNA
- c) change of a single nucleotide in DNA
- d) tandem repeat of a short motif

27. Which enzyme is used in PCR to synthesize DNA at high temperatures?

- a) DNA polymerase III
- b) reverse transcriptase
- c) Taq polymerase
- d) RNase H

28. What does the Ct value in quantitative PCR indicate?

- a) template concentration in mg/ml
- b) the number of cycles after which fluorescence is detected
- c) length of amplified fragment
- d) denaturation temperature

29. What is the main difference between Sanger sequencing and NGS?

- a) Sanger sequencing uses RNA template
- b) NGS allows parallel sequencing of many fragments
- c) Sanger sequencing uses fluorescent nucleotides; NGS does not
- d) NGS is less accurate than Sanger sequencing

30. What is the principle of DNA microarray?

- a) DNA amplification
- b) hybridization of analyzed DNA or RNA with probes
- c) sequencing using dideoxynucleotides
- d) protein visualization with ethidium bromide

31. What do DNA microarrays allow?

- a) gene expression analysis
- b) RNA sequencing
- c) protein production
- d) primer synthesis

32. What is the main principle of real-time PCR?

- a) monitoring fluorescence in each PCR cycle, which increases proportionally to product amount
- b) using radioactively labeled nucleotides to visualize PCR fragments
- c) amplification of RNA without reverse transcription
- d) sequencing PCR products during synthesis

33. What does genomics study?

- a) protein function
- b) the complete set of DNA of an organism
- c) metabolic pathways
- d) RNA expression

34. Which technique is often used for whole-genome sequencing?

- a) Western blot
- b) mass spectrometry
- c) next-generation sequencing
- d) PCR

35. Which term refers to all genes of an organism?

- a) proteome
- b) genome
- c) metabolome
- d) transcriptome

36. Which method is used to identify associations between genetic variants and traits or diseases?

- a) GWAS (genome-wide association studies)
- b) RNA sequencing
- c) ELISA
- d) flow cytometry

37. Transcriptomics studies:

- a) DNA sequences
- b) RNA molecules expressed in cells
- c) metabolites in blood
- d) viruses

38. Which technique is fundamental for transcriptomic studies?

- a) RNA sequencing
- b) CRISPR-Cas9
- c) ELISA
- d) flow cytometry

39. Which type of RNA is most often analyzed in transcriptomics?

- a) rRNA
- b) mRNA
- c) tRNA
- d) miRNA

40. Which term refers to all RNA molecules in a cell?

- a) proteome
- b) genome
- c) transcriptome
- d) metabolome

41. Metabolomics studies:

- a) DNA variations
- b) protein expression
- c) small molecules/metabolites in cells and tissues
- d) viruses

42. Which analytical methods are commonly used in metabolomics?

- a) mass spectrometry and NMR
- b) Sanger sequencing
- c) Northern blot
- d) immunohistochemistry

43. Which term refers to the study of a total set of metabolites in a cell or organism?

- a) genome
- b) proteome
- c) metabolome
- d) transcriptome

44. Which research field integrates metabolomics with genomics and transcriptomics?

- a) proteomics
- b) multi-omics
- c) viromics
- d) epigenomics

45. Viromics studies:

- a) the entire human genome
- b) viral populations and their genetic diversity
- c) RNA expression in bacteria
- d) metabolites in blood

46. Which technique is key for viromics?

- a) viral metagenomics
- b) ELISA
- c) Western blot
- d) flow cytometry

47. Which term refers to all viruses in a particular environment or host?

- a) virome
- b) viromic
- c) genome
- d) proteome

48. Which area of viromics studies virus-host interactions?

- a) host-virus interactome
- b) proteomics
- c) metabolomics
- d) genomics

49. What is the goal of functional genomics?

- a) to study interactions between genes
- b) to predict protein 3D structure
- c) to analyze metabolic pathways
- d) to identify viral populations

50. Which method is used to compare the genomes of different organisms?

- a) Comparative genomics
- b) RNA sequencing
- c) Western blot
- d) Flow cytometry

51. The term proteome refers to the set of all:

- a) proteins of a cell
- b) RNA molecules
- c) genes
- d) metabolites

52. What does proteomics enable in addition to genomics?

- a) determination of which proteins are actually expressed
- b) determination of the DNA sequence
- c) detection of all metabolites
- d) identification of all RNA transcripts

53. What is characteristic of the transcriptome?

- a) is dynamic and changes depending on conditions
- b) is identical in all cells
- c) includes all DNA sequences
- d) contains only rRNA

54. What is the main difference between the proteome and transcriptome?

- a) The proteome is stable, the transcriptome is dynamic
- b) The transcriptome reflects RNA, the proteome reflects proteins
- c) The proteome is unrelated to gene expression
- d) The transcriptome includes metabolites

55. Which technology is used to map the expression of thousands of genes at once?

- a) DNA microarray
- b) ELISA
- c) Western blot
- d) PCR

56. Which omics field is most closely associated with personalized medicine?

- a) Proteomics
- b) Genomics
- c) Metabolomics
- d) Viromics

57. What type of data is generated in metabolomics?

- a) Nucleotide sequences
- b) Spectral profiles
- c) Protein maps
- d) RNA reads

58. Viromics:

- a) studies only viruses with DNA genomes
- b) characterizes all viruses in an environment
- c) analyzes viral proteins
- d) detects host metabolites

59. Which field specifically studies epigenetic changes in the genome?

- a) Epigenomics
- b) Proteomics
- c) Viromics
- d) Metabolomics

60. Why is a multi-omics approach important?

- a) integrates multiple layers of biological information
- b) replaces all experimental methods
- c) focuses only on RNA
- d) includes only proteins

61. What is the main advantage of NMR in metabolomics?

- a) High sensitivity
- b) Non-invasive analysis
- c) DNA sequencing
- d) RNA quantification

62. What areas does systems biology study?

- a) Only metabolomics
- b) Complex signaling pathways among genes, proteins, and metabolites
- c) Only DNA replication
- d) Only viromics

63. What does epigenomics study?

- a) DNA sequence
- b) Gene mutations
- c) Chemical modifications of DNA and histones affecting gene expression
- d) Only mitochondrial DNA

64. Which discipline analyzes networks of interactions between proteins?

- a) Lipidomics
- b) Glycomics
- c) Interactomics
- d) Pharmacogenomics

65. What is the main subject of lipidomics?

- a) Glycosylation of proteins
- b) The composition and function of lipids in cells
- c) Expression of microRNAs
- d) Tandem DNA repeats

66. The goal of glycomics is to:

- a) study the structure and function of carbohydrates and glycans
- b) analyze mutations in rRNA
- c) measure metabolite levels
- d) study pathogenic microorganisms

67. Which field focuses on the composition and function of the gut microbiome?

- a) Epigenomics
- b) Microbiomics
- c) Exosomics
- d) Pharmacogenomics

68. What does pharmacogenomics study?

- a) Genetic factors influencing response to treatment
- b) Gene expression in infections
- c) Metabolic products of cells
- d) Interactions between proteins

69. What is the subject of exosomics?

- a) Analysis of histone modifications
- b) The content of vesicles carrying nucleic acids, proteins, and lipids
- c) Monitoring of protein glycosylation
- d) Analysis of extracellular vesicles as biomarkers

70. Which type of molecules is the main subject of glycomics research?

- a) Lipids
- b) Carbohydrates and glycan structures
- c) RNA transcripts
- d) Phosphoproteins

Answer Key to Model Questions

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|----------|----------|----------|
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| 7. FFTF | 31. TFFF | 55. TFFF |
| 8. FTFF | 32. TFFF | 56. FTTF |
| 9. FTFF | 33. FTFF | 57. FTFF |
| 10. FTFF | 34. FFTF | 58. FTFF |
| 11. FTFF | 35. FTFF | 59. TFFF |
| 12. TTFF | 36. TFFF | 60. TFFF |
| 13. FFTF | 37. FTFF | 61. FTFF |
| 14. FFTF | 38. TFFF | 62. FTFF |
| 15. FTFF | 39. FTFF | 63. FFTF |
| 16. TTFF | 40. FFTF | 64. FFTF |
| 17. TFFF | 41. FFTF | 65. FTFF |
| 18. FTFF | 42. TFFF | 66. TFFF |
| 19. TFFF | 43. FFTF | 67. FTFF |
| 20. FTFF | 44. FTFF | 68. TFFF |
| 21. FTFF | 45. FTFF | 69. FTFT |
| 22. FTFF | 46. TFFF | 70. FTFF |
| 23. FTFF | 47. TFFF | |
| 24. TFFF | 48. TFFF | |

12 Forms of entry and existence of foreign DNA in the cell. Vectors. Recombinant techniques

Foreign DNA can enter a cell under certain circumstances, either naturally or through specialized laboratory methods, and subsequently exist in cells in different forms. The natural way for DNA to enter a bacterial cell is through **transformation**, i.e., the uptake of free DNA from the environment; however, the bacterial cell must be prepared for transformation, a process known as competence. This phenomenon was first demonstrated by Griffith in pneumococci, and today we know that some bacteria possess specialized transport systems that enable them to incorporate foreign DNA fragments into their own genome. Bacterial transformation can also be artificially induced in the laboratory, which is widely used in the creation of new GMO bacteria in genetic engineering.

Another mechanism, occurring naturally and also exploited in laboratories, is **transduction**, in which genetic transfer is mediated by viruses, most commonly bacteriophages and, in eukaryotic systems, retroviruses. During the viral replication cycle, genetic material from a previous host may be introduced into a new cell, resulting in horizontal gene transfer. A third mechanism is **conjugation**, which requires direct contact between two bacterial cells through specialized projections called pili. This process mainly transfers plasmids, which often carry genes associated with antibiotic resistance. During **viral infection**, the virus introduces its nucleic acid genome into a host cell, where it can direct viral replication and the production of new virions.

In genetic engineering, special methods are used that mimic or bypass natural mechanisms. **Microinjection** represents a direct intervention in which DNA is introduced into the cell nucleus using a micropipette, employed especially in manipulations with oocytes and embryos. **Electroporation** relies on a short electric pulse that temporarily increases the permeability of the cytoplasmic membrane, thereby allowing DNA to enter the cell. Similar effects are achieved with **chemical methods**, where compounds such as CaCl_2 disrupt the membrane, or with lipofection, where DNA is encapsulated into liposomes that then fuse with the membrane. In plant biotechnology, **biolistics** (the “gene gun”) is used, shooting microscopic particles of gold or tungsten coated with DNA into cells. In biomedicine, **viral vectors** are genetically modified retroviruses, adenoviruses, or adeno-associated viruses that have been stripped of their pathogenic properties while retaining the ability to efficiently deliver foreign DNA into target cells.

Forms of Existence of Foreign DNA in the Cell

After entering a cell, foreign DNA may exist in various forms. The simplest is the **plasmid form**, in which DNA remains outside the chromosomes as an independent circular molecule capable of autonomous replication. In bacteria, this is a common phenomenon, and plasmids often carry genes that confer resistance or facilitate metabolic pathways. A similar state is the

episomal form, where DNA remains outside the main chromosome but is capable of coordinated replication with the host genome. **Episomes** are found, for example, in some viruses or specific plasmids. The most stable variant is the **integrated form**, in which foreign DNA is directly incorporated into the host chromosome. This state is typical for retroviruses, which, after reverse transcription of their RNA genome, integrate the DNA into the cell genome as a provirus. Transposon activity also belongs in this category. Conversely, some DNA fragments may exist only transiently, lacking the ability to replicate. Such a **transient form** disappears after a few cell divisions because the DNA neither integrates nor replicates autonomously, but rather functions temporarily, for example, during gene transfer under laboratory conditions.

Vectors

Vectors are specialized carriers of genetic information that enable the transfer of foreign DNA into cells. In biology and medicine, they are primarily used in genetic engineering and gene therapy, where they play a crucial role in manipulating genetic material. Vectors must be able to deliver DNA into target cells, allow its replication or expression, and at the same time avoid causing serious adverse effects.

The oldest and still widely used type are **plasmid vectors**. Plasmids are small, circular DNA molecules found in bacteria that are capable of independent replication. In the laboratory, they are modified to carry the desired gene, along with regulatory sequences such as promoters or selection markers. They are mainly used in microbiology and biotechnology for gene cloning or protein production.

Viral vectors are also of great importance. Their natural property is the ability to enter cells, deliver genetic information, and, in some cases, integrate their genetic material, a process that is exploited in gene therapy. **Retroviruses** can permanently integrate a foreign gene into the host genome but carry the risk of mutagenic effects (insertional mutagenesis). **Adenoviruses**, in contrast, deliver DNA without integration into the chromosome, which is advantageous for temporary gene expression. **Adeno-associated viruses (AAV)** are used for their high safety and low immunogenicity.

Besides viral, there are also **non-viral vectors**. These include liposomes or lipid nanoparticles, which encapsulate DNA and enable its entry into the cell by fusing with the membrane. Such systems are less efficient than viral ones but safer, as they do not carry the risk of mutation or immunogenicity. This category also includes synthetic polymers and inorganic nanoparticles. A special group of bacterial vectors uses attenuated pathogens (e.g., *Salmonella*, *Listeria*) that naturally enter cells and can transfer recombinant DNA. Such approaches are being tested, for example, in the development of vaccines.

Principle of Recombinant Techniques

The basis of recombinant DNA technology lies in the use of restriction endonucleases, DNA ligase, a suitable vector, and the desired gene. The DNA with the target gene and the vector (plasmid, viral genome) are separately cut with compatible restriction enzymes, producing so-called “sticky” or blunt ends, which are then joined by ligase into a recombinant molecule. The vector contains an origin of replication, a selection marker, and often regulatory elements for expression. The recombinant DNA is then introduced into the host via transformation in bacteria, or transfection/transduction in eukaryotes. The resulting clones are selected and verified.

Modern methods also use PCR, which allows amplification, the addition of specific restriction sites, and precise “stitching” of fragments (e.g., Gibson assembly, Golden Gate). For targeted gene repair, homologous recombination (HDR) with a supplied template is applied, or non-homologous end joining (NHEJ), which often leads to frame-shift deletions and “knock-outs.” Programmable nucleases such as **ZFNs, TALENs, and CRISPR-Cas9/12** create a double-strand break at a precisely defined sequence; the outcome depends on the chosen repair pathway (HDR vs. NHEJ) and the design of the donor template.

Cut-free editing employs “**base editors**” (correction of a single base/nucleotide) and “**prime editors**” (correction of short sequences), which allow the modification of single bases or small DNA segments without creating double-strand breaks, thereby reducing the risk of off-target effects. In clinical applications, the editing machinery is delivered in the form of mRNA or protein-RNP complexes, or transiently via AAV or lentiviruses. Great emphasis is placed on precise tissue targeting (specific promoters, AAV serotypes, ligand-coated nanoparticles) and minimizing side effects.

Basic Methods of Gene Therapy

In cases of mutations leading to the loss of a gene/gene product, the most common strategy is to deliver a functional copy of the gene to replace the missing or defective information. In gene repair, also known as **gene editing**, the precise error in the DNA is corrected, ideally without inserting foreign sequences. For cancer therapy, targeted cytotoxicity is used either by expressing a so-called “suicide gene,” which converts a non-toxic prodrug/precursor into a toxic product inside the cell, or by stimulating the immune system against the tumor, for example, with **CAR-T cells**. Gene silencing, also known as knockdown, can be achieved using antisense oligonucleotides or **RNA interference (RNAi)** mechanisms. In modern variants, **CRISPR-i** (CRISPR interference) and **CRISPR-a** (CRISPR activation), a catalytically inactive Cas protein (dCas = dead Cas) is fused with a repressor or activator, thereby turning a gene off or on in a targeted manner.

CRISPR-Cas System

This is a natural antiviral mechanism of bacteria and archaea that provides adaptive immunity against bacteriophages as well as plasmids. It is based on specific clustered regularly interspaced short palindromic repeats (**CRISPR**) in the genome and associated CRISPR-associated (Cas) genes, which encode Cas proteins. During the first infection, the bacterium incorporates a short fragment of viral DNA (called a spacer) into the CRISPR locus. This sequence is then transcribed into a short **crRNA**, which, together with a Cas protein, recognizes the corresponding sequence during reinfection and specifically degrades it. The most well-known is the **Cas9** protein, which enables precise DNA cleavage; other variants also exist. **Cas12** targets DNA, while **Cas13** is specific for RNA. The fundamental principle of CRISPR-Cas is often described as a **two-component system**, where crRNA provides sequence specificity and the Cas protein performs the cleavage of the target nucleic acid. This mechanism provides bacteria with adaptive immune memory and, at the same time, has become the foundation of modern genetic engineering.

Gene Therapy

Gene therapy is a treatment for diseases that involves interventions in DNA/RNA, with the goal of delivering a functional gene into target cells, repairing a mutation, or silencing the expression of a harmful gene/allele. Most monogenic disorders arise from reduced or lost gene function; therefore, the therapeutic aim is straightforward: deliver the correct sequence into the correct cells at the correct time in a permanently safe manner. Clinically, the main approaches are “add, replace, silence.” In the “add” approach, a copy of the gene is introduced; in the “replace” approach, the defective sequence is edited back to its physiological version; and in the “silence” approach, the expression of the pathogenic version is blocked (at the level of DNA, RNA, or protein).

The first regulatory approvals paved the way for routine clinical use. For example: *Luxturna* targets mutations in the **RPE65** gene in inherited retinopathy, *Zolgensma* delivers a functional copy of the **SMN1** gene in spinal muscular atrophy, and *Strimvelis* provides a functional **ADA** gene for the missing ADA enzyme in a form of SCID. In oncology, genetically modified cells (CAR-T; *Kymriah*, *Yescarta*) and oncolytic viruses (*Imlygic*) have also been applied. The common denominator of success is a clear causal link between mutation and phenotype, accessibility of suitable target cells, and a reliable delivery system.

Key requirements include precise molecular diagnosis, including sequencing and validation of variant pathogenicity; selection of target cells with long-term renewal potential, ideally autologous stem cells; use of a safe and efficient vector; and control of gene expression. **Viral** vectors, including adeno-associated viral, lentiviral, adenoviral, and retroviral vectors, remain the most effective delivery systems. **Non-viral** approaches include naked DNA, lipid- and polymer-based nanoparticles, and physical methods such as electroporation. Therapy can be conducted *in vivo*, by delivering the therapeutic construct directly into the target tissue, or *ex*

vivo, by modifying isolated cells followed by reinfusion into the patient. The *ex vivo* approach offers better control over cell modification and selection, whereas the *in vivo* approach is more practical for tissues that cannot be safely removed, expanded, and reinfused.

The advantages of gene therapy lie in its causal nature and the potential for one-time, long-lasting effects. Limitations include vector immunogenicity, random integration with the risk of insertional mutagenesis, limited vector capacity, transient expression in non-integrating systems, the complexity of polygenic diseases, therapy cost, and thus limited accessibility for all patients with a given condition. Safety is paramount: preventing vector recombination, avoiding off-target effects (outside the target organ/tissue), and respecting ethical boundaries (in humans, strictly somatic, not germline therapy).

The table below provides an overview of the principal forms of gene therapy.

Type of Intervention	Principle	Techniques / Tools
Gene addition	A functional copy of the missing gene is delivered into the cell	Recombinant vectors (AAV, retro/lenti), plasmids, liposomes
Gene replacement / repair	The mutated sequence is cut out and replaced with a healthy sequence	CRISPR-Cas9, TALEN, ZFN; HDR with donor template
Gene silencing (ablation)	Suppression of pathogenic gene expression	RNAi, antisense oligonucleotides, CRISPR-i
Suicide gene therapy	A “killer” gene is introduced into cells, activated by a drug or producing a toxin	HSV-thymidine kinase + ganciclovir; “suicide gene therapy”
Immunogene therapy	Genetically modified cells or viruses stimulate the immune system against the tumor	CAR-T cells, oncolytic viruses (modified HSV, adenovirus)
Mitochondrial therapy	Replacement of mutated mitochondrial genes	Nuclear transfer into a donor oocyte, mitochondrial transfers



Model questions

1. Natural ways of foreign DNA entering a cell are:

- a) transformation
- b) transcription
- c) transduction
- d) conjugation

2. Experimental techniques of DNA transfer into a cell include:

- a) electrophoresis
- b) microinjection
- c) lipofection
- d) photosynthesis

3. Viral vectors used in genetic engineering may be:

- a) retroviruses
- b) chloroplasts
- c) adenoviruses
- d) mitochondria

4. Foreign DNA can exist in a cell as:

- a) plasmid form
- b) mitochondrial form
- c) episomal form
- d) ribosomal form

5. The integrated form of foreign DNA is typical for:

- a) retroviruses
- b) plasmids
- c) transposons
- d) liposomes

6. Plasmid vectors:

- a) are small circular DNA molecules capable of autonomous replication
- b) are naturally found in the nuclei of eukaryotic cells
- c) may carry selection markers and promoters
- d) are never used for protein production

7. What is true about viral vectors?

- a) retroviruses integrate into the host genome
- b) adenoviruses deliver DNA without integration
- c) adeno-associated viruses are highly immunogenic
- d) all viral vectors are always pathogenic

8. Non-viral vectors:

- a) include liposomes and lipid nanoparticles
- b) are less safe than viral vectors
- c) may be made of synthetic polymers
- d) are used only in plant biotechnology

9. Bacterial vectors:

- a) use attenuated pathogens, such as Salmonella and Listeria
- b) do not allow transfer of recombinant DNA
- c) are tested in vaccine development
- d) are already completely replaced by viral vectors

10. Recombinant DNA technology:

- a) uses restriction endonucleases and DNA ligase
- b) allows insertion of a foreign gene into a vector
- c) is based on transcription into mRNA
- d) does not allow clone selection

11. Programmable nucleases:

- a) ZFN, TALEN, and CRISPR-Cas can create a double-strand break in DNA
- b) the outcome of editing depends on the chosen repair pathway, HDR or NHEJ
- c) use only retroviruses
- d) belong exclusively to bacterial enzymes without use in genetics

12. Base editors and prime editors:

- a) allow base or short DNA sequence changes without double-strand breaks
- b) increase the risk of off-target effects
- c) represent modern variants of precise editing
- d) always require integration into the host chromosome

13. In mutations causing loss of gene function, gene therapy most often:

- a) removes the entire chromosome
- b) delivers a functional copy of the gene
- c) uses RNAi
- d) edits the exact error in DNA

14. Targeted cytotoxicity in gene therapy may occur through:

- a) expression of a “suicide” gene, leading to activation of a prodrug/precursor
- b) expression of a gene for antibiotic resistance
- c) activation of the immune system using CAR-T cells
- d) expression of the gene for GFP (green fluorescent protein)

15. Gene silencing (knockdown) is achieved by:

- a) antisense oligonucleotides
- b) RNAi mechanism
- c) CRISPR-x/CRISPR-a system
- d) transcription of the gene into mRNA

16. The CRISPR-Cas system in bacteria:

- a) provides adaptive immunity against bacteriophages
- b) uses short fragments of viral DNA stored as spacers
- c) degrades the host cell RNA
- d) was discovered as an archaeal defense against plasmids in 2020

17. The best-known Cas proteins:

- a) Cas9 enables precise DNA cleavage
- b) Cas9 degrades proteins
- c) Cas12 and Cas13 function exclusively against RNA viruses
- d) Cas12 and Cas13 target DNA and RNA, respectively

18. The principle of gene therapy consists mainly of:

- a) introducing a functional gene into target cells
- b) permanently silencing all mutated genes in the body
- c) repairing a mutation or silencing a harmful allele
- d) replacing the entire nucleus of the cell

19. The first regulatory-approved therapies include:

- a) Luxturna for mutations in RPE65
- b) Zolgensma for spinal muscular atrophy
- c) penicillin as the first antibiotic
- d) chemotherapy based on the cytostatic cisplatin

20. Key requirements for successful gene therapy are:

- a) precise molecular diagnosis
- b) selection of suitable target cells
- c) delivery of the gene into cytoplasmic inclusions
- d) complete elimination of host RNA

21. Ex vivo gene therapy:

- a) means modification of isolated cells outside the body and their return to the patient
- b) allows selection and control of modified cells
- c) is more practical for tissues that cannot be harvested
- d) always occurs directly inside the patient's body

22. Among the limitations of gene therapy are:

- a) immunogenicity of vectors
- b) risk of insertional mutagenesis
- c) long-term effect with integrating systems
- d) the ability to solve all polygenic diseases

23. CRISPR/Cas is:

- a) a two-component immune system of eukaryotic cells
- b) a naturally occurring adaptive two-component immune system of prokaryotic organisms
- c) a naturally occurring adaptive two-component immune system of viruses
- d) a two-component immune system of prokaryotic cells located in mitochondria or chloroplasts

24. CRISPR/Cas:

- a) is a type of plasmid
- b) is a system by which bacteria defend themselves against foreign DNA
- c) nonspecifically cleaves invading protein
- d) nonspecifically cleaves invading nucleic acid

25. Potential applications of CRISPR/Cas in medical practice include:

- a) cancer therapy
- b) treatment of monogenic diseases
- c) treatment of viral infections
- d) addressing antibiotic resistance

26. Anti-CRISPR (Acr) proteins:

- a) block DNA binding sites
- b) block DNA cleavage
- c) enzymatically deactivate CRISPR/Cas
- d) are transferred by plasmids

27. CRISPR-based antibiotics:

- a) cannot be targeted at plasmids responsible for bacterial resistance
- b) have a broad-spectrum effect
- c) can be targeted directly against bacterial genomes
- d) inhibit bacterial cell wall synthesis

28. Gene therapy:

- a) is somatic or germline
- b) is largely banned in humans for ethical reasons
- c) can be in vivo or ex vivo
- d) uses genetic material to treat or prevent disease

29. Somatic gene therapy:

- a) uses viral vectors for gene transduction
- b) uses viral vectors for gene transfection
- c) uses plasmids for gene transfection
- d) uses plasmids for gene transduction

30. RNA therapy:

- a) uses DNA-based molecules for disease treatment
- b) uses antisense oligonucleotides
- c) can be used to deliver mRNA into cells to restore a missing enzyme
- d) does not use aptamers

31. A vector in gene therapy:

- a) means a gene delivery system
- b) is always integrated into the host genome
- c) can be derived from a virus by deleting viral genes
- d) depending on type, may integrate into the host genome

32. A plasmid can confer:

- a) resistance to antibiotics, chemotherapeutics, and heavy metals
- b) replication of the bacterial genome
- c) production of antibiotics and toxins
- d) degradation and oxidation of otherwise biologically inert or toxic organic compounds

33. A plasmid can confer:

- a) production of restriction and modification enzymes
- b) formation of the bacterial nuclear membrane
- c) ability to engage in symbiosis
- d) synthesis of bacterial membrane organelles

34. Transfer of a plasmid from cell to cell:

- a) is divided into horizontal and vertical
- b) horizontal transfer also occurs between different species and genera of bacteria
- c) vertical transfer occurs between different species and genera of bacteria
- d) also occurs during amitosis

35. Horizontal transfer of a plasmid from cell to cell:

- a) occurs during amitosis from parent to daughter cells
- b) occurs only between the same bacterial genera
- c) is responsible for interspecies and intergenus transfer of antibiotic resistance
- d) occurs via conjugation

36. Diseases suitable for gene therapy are:

- a) Down syndrome
- b) malignancies
- c) severe combined immunodeficiency – SCID
- d) Duchenne muscular dystrophy

37. Cloning is used to prepare:

- a) enzymes
- b) hormones
- c) vaccines
- d) blood factors

38. Cosmids are vectors obtained by combining:

- a) viruses and human DNA
- b) plasmids and phages
- c) phages and proteins
- d) YACs and yeast

39. Cosmids are:

- a) antibiotics
- b) vectors
- c) cloning sites
- d) enzymes

40. According to basic function, vectors are divided into:

- a) cloning
- b) expression
- c) localized
- d) multifunctional

41. Retroviral vectors in γ -retroviral gene therapy can infect:

- a) non-dividing cancer cells
- b) only dividing cells
- c) dividing and non-dividing cells
- d) only non-dividing cells

42. Recombinant DNA:

- a) cannot be produced artificially
- b) standard abbreviation is rDNA
- c) is DNA that contains two or more sequences that do not normally occur together
- d) cannot occur naturally

43. Recombinant DNA technology:

- a) is a set of methods for creating recombinant DNA
- b) is not a technology for DNA production
- c) can modify DNA in living organisms
- d) includes cloning techniques

44. Recombinant DNA technology is:

- a) useful for producing recombinant proteins (e.g., insulin, growth hormone)
- b) prohibited
- c) unnecessary
- d) used in gene therapy

45. Foreign DNA can enter a bacterial cell via:

- a) transduction
- b) sexduction
- c) malporation
- d) transformation

46. Foreign DNA can also enter a bacterial cell via:

- a) transduction
- b) fortification
- c) conjugation
- d) malporation

47. Foreign DNA can enter a eukaryotic cell via:

- a) transposons
- b) fortification
- c) electroporation
- d) transduction

48. Bacterial resistance to antibiotics can be caused by:

- a) hyperplasia
- b) presence of an antibiotic resistance gene in the bacterium
- c) presence of a plasmid
- d) presence of a transposon

49. Plasmid:

- a) is double-stranded circular DNA
- b) is linear single-stranded RNA
- c) can confer an advantage to the bacterium
- d) is found only in eukaryotic cells

50. Plasmid replication:

- a) depends on the bacterial chromosome
- b) is independent of the bacterial chromosome
- c) occurs via the “rolling circle” mechanism
- d) occurs via the “rolling stone” mechanism

51. Plasmid – additional properties:

- a) can serve as a vector for gene transfer
- b) is linear single-stranded RNA
- c) can carry an antibiotic resistance gene
- d) is located in the eukaryotic nucleus

52. Transposons:

- a) arise only artificially in the laboratory
- b) are DNA elements that can move within or between genomes
- c) are not present in all genomes
- d) can act as natural mutagens

53. Transposons:

- a) are present in every genome
- b) arise only artificially in the laboratory
- c) can carry antibiotic resistance genes
- d) can act as natural mutagens

54. Applications of recombinant DNA technology:

- a) medicine
- b) biotechnology
- c) research
- d) environmental sciences

55. Conjugation:

- a) is the transfer of genetic material between two bacterial cells
- b) occurs via a pilus
- c) is the transfer of genetic material between two multicellular eukaryotic organisms
- d) starts with the recipient cell forming a pilus toward the donor cell

56. Which mechanisms allow natural DNA transfer between bacteria?

- a) transformation
- b) transduction
- c) conjugation
- d) translocation

57. Which laboratory methods are used to introduce foreign DNA into a cell?

- a) replication
- b) electroporation
- c) lipofection
- d) microinjection

58. Which statements about forms of foreign DNA in a cell are correct?

- a) plasmid form prevents autonomous replication
- b) episomal form replicates coordinately with the genome
- c) integrated form means DNA is incorporated into the chromosome
- d) transient form becomes a permanent part of the genome

59. Which types of vectors are used in genetic engineering and therapy?

- a) plasmid
- b) viral (staphylococci, streptococci)
- c) lipid nanoparticles
- d) ribosomes

60. Which statements about the CRISPR-Cas system are correct?

- a) provides bacteria with adaptive immunity against adenoviruses
- b) uses short segments of viral DNA stored in the CRISPR locus
- c) the Cas9 protein allows precise DNA cleavage
- d) was originally a translation mechanism

Answer Key to Model Questions

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| 8. TFTF | 28. TFTT | 48. FTTT |
| 9. TFTF | 29. TFTF | 49. TFTF |
| 10. TTFF | 30. FTTF | 50. FTTF |
| 11. TTFF | 31. TFTT | 51. TFTF |
| 12. TFTF | 32. TFTT | 52. FTFT |
| 13. FTFF | 33. TFTF | 53. TFTT |
| 14. TFTF | 34. TTFT | 54. TTTT |
| 15. TTFF | 35. FFTT | 55. TTFF |
| 16. TTFF | 36. FTTT | 56. TTTF |
| 17. TFFT | 37. TTTT | 57. FTTT |
| 18. TFTF | 38. FTFF | 58. FTTF |
| 19. TTFF | 39. FTFF | 59. TFTF |
| 20. TTFF | 40. TTFF | 60. FTTF |

13 Relationships between micro- and macroorganisms

Both unicellular and multicellular organisms live within networks of relationships that shape their life processes. In humans, we distinguish between indifferent relationships (where many microbes do not affect us at all), the stable coexistence of commensals and symbionts on the skin, mucosal surfaces, and in the gut, as well as pathogenic interactions that lead to disease. The balanced composition of the microbiota is referred to as **eubiosis**. In contrast, its disturbance is **dysbiosis** (which is, for example, associated with inflammatory diseases of the large intestine, obesity, autoimmune disorders, and abnormalities in nervous system development).

Pathogenicity refers to a microorganism's ability to cause disease. The course of infection depends on the virulence of the pathogen, the **infectious dose**, and the **immune status** of the host. **Obligate pathogens** (e.g., *Vibrio cholerae*, *Salmonella Typhi*) cause disease in nearly all infections, whereas **opportunistic pathogens** cause disease primarily when immunity is impaired or after entry into an unusual niche (e.g., *E. coli* in the urinary tract).

Prevalence of a particular parasite in a population (the number of infected individuals) is an important epidemiological indicator that explains local differences in morbidity and enables effective assessment of preventive measures and the need for targeted interventions in a given area.

Bacteria

Bacteria are unicellular prokaryotic organisms that are markedly smaller than human eukaryotic cells (most measure approximately 0.2–2 μm). Their morphology is diverse. **Cocci**, i.e., spherical bacteria, may occur as single cells, **pairs** (diplococci), **chains** (streptococci), or **grape-like clusters** typical of staphylococci. **Bacilli**, i.e., rod-shaped bacteria, may be straight or curved, and some form chains. **Vibrios** have a shape that resembles curved, comma-like cells, as seen in *Vibrio cholerae*. **Spirilla** and **spirochetes** are spiral bacteria; spirochetes are thin, long, and highly motile, for example, *Treponema pallidum*. In addition, there are **filamentous** bacterial forms that form thread-like structures, such as *Actinomyces*. Some bacteria are capable of forming highly resistant **spores** with minimal water content and dormant metabolism, which allows them to survive extreme environmental conditions.

The relationship of bacteria to oxygen is strongly species-specific. Besides **obligate aerobes** and **obligate anaerobes**, there are **facultative anaerobes**, **microaerophiles**, and **aerotolerant anaerobes**. Bacterial reproduction ordinarily proceeds **asexually (binary fission)**, producing clonal populations. Genetic diversity is ensured by horizontal gene transfer, which includes transformation, transduction, and conjugation. Plasmids are small, autonomously replicating replicons and may be transmitted vertically during cell division as well as horizontally by conjugation (and also by transformation and transduction). Plasmids often encode antibiotic

resistance, heavy metal resistance, toxins, virulence factors, restriction modification systems, or metabolic pathways (e.g., degradation of aromatic compounds or pesticides).

A natural defensive arsenal of many bacteria is the **adaptive CRISPR/Cas system**, which recognizes and cleaves foreign nucleic acids. Its activity can be attenuated by **anti-CRISPR proteins** that block binding or catalytic function. In the diagnostics of antimicrobial resistance, beyond phenotypic tests, **genotypic methods** are used that predict the presence of specific genes (not the magnitude of their effect). These methods can also detect new variants of resistance determinants. In clinical practice, simple microscopy, combined with Gram staining, remains important.

The **human microbiota** naturally colonizes both the skin and the gut. In the large intestine, in addition to *E. coli*, we also find fermentative and putrefactive non-pathogenic bacteria that play important roles in digestion and in maintaining internal homeostasis. The blood of a healthy individual is sterile. Some bacteria are the causative agents of serious diseases. These include tuberculosis caused by *Mycobacterium tuberculosis*, tetanus caused by *Clostridium tetani*, botulism caused by *Clostridium botulinum*, respiratory infections, and severe diarrheal diseases provoked by bacteria such as *Salmonella*, *Shigella*, or enteropathogenic strains of *E. coli*. Among the best-known sexually transmitted bacterial infections are syphilis (*Treponema pallidum*) and gonorrhea (*Neisseria gonorrhoeae*). The toxin produced by *Clostridium botulinum* – botulinum toxin – is among the most potent toxins known; it blocks neuromuscular transmission and leads to severe paralysis and even death. Like many other compounds, botulinum toxin can be used in controlled, extremely low doses, for example, to treat muscle spasms, or in aesthetic medicine to smooth wrinkles.

Parasitic protozoa (protozoans)

Protozoa are eukaryotic, heterotrophic unicellular organisms that often have complex life cycles and may use multiple hosts. The cell surface may bear flagella or cilia, an undulating membrane may be present, and movement can also occur via pseudopodia. Many protozoa are able to form **anabiotic forms** (resistant resting stages), such as **cysts**, which allow them to persist for long periods under unfavorable conditions. Diagnosis relies on microscopy of native wet-mount or stained preparations and is complemented by immunological and molecular methods. Parasitic protozoa can be classified according to their main **site of infection** into intestinal and other luminal forms, blood forms, and tissue forms.

A typical representative of **luminal protozoa** is *Trichomonas vaginalis*, which is one of the largest human flagellates. It causes urogenital trichomoniasis – discharge in women after approximately 10 days of incubation, and in men, often latent urethritis. The organisms are pear-shaped (15–30 µm), with four free flagella, a fifth incorporated into an undulating membrane, and a longitudinal axial rod (axostyle). Transmission is by sexual intercourse. *T. vaginalis* is not transmitted by blood or by droplets but exclusively through intimate contact, making it a prototypical sexually transmitted protozoan pathogen.

An example of an intestinal **parasitic protozoan** is *Entamoeba histolytica*. It is one of the markedly pathogenic amoebae of humans, causing amoebic dysentery with bloody diarrhea, painful cramping, and a risk of extraintestinal abscesses. It occurs as a non-invasive *minuta* form and an invasive *magna* form. It forms resistant quadrinucleate cysts and is transmitted by the fecal-oral route. Invasion of the colonic mucosa leads to amoebiasis (formation of broad, bleeding ulcers on the colonic mucosa).

Another example is *Giardia intestinalis* – a flagellate that parasitizes the small intestine. It excysts in the duodenum, attaches via a ventral sucking disk, and often causes a malabsorption syndrome characterized by pale, greasy, mucous stools. Diagnosis is based on the detection of trophozoites in duodenal aspirate or on antigen/DNA tests.

Among the blood and tissue protozoa is *Toxoplasma gondii*, a very widespread parasite of humans and animals. Several developmental stages are recognized in its life cycle. **Tachyzoites** are rapidly dividing, virulent forms that disseminate during the acute phase of infection, causing cell damage. **Bradyzoites** are slowly dividing forms that persist inside tissue cysts, especially in the brain, skeletal muscles, lungs, and liver. In these sites, *Toxoplasma* persists in a so-called latent state – that is, in the chronic phase of infection.

The definitive host is the cat and other felids, which shed oocysts into the environment; these are highly resistant and capable of long-term survival. In immunocompetent individuals, toxoplasmosis is usually asymptomatic, whereas in immunodeficient patients it can lead to severe complications. Infection can also be transmitted transplacentally, causing congenital toxoplasmosis with serious consequences for the fetus. Another example is the plasmodia (e.g., *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*), which cause malaria. Following the transmission of sporozoites by a female Anopheles mosquito, a hepatic phase is followed by an erythrocytic phase of the disease, which produces periodic febrile paroxysms. *P. vivax* and *P. ovale* can persist as hypnozoites and cause relapses.



Model questions

1. Bacteria are:

- a) acellular pathogens of humans
- b) cellular pathogens of humans
- c) eukaryotic microorganisms
- d) prokaryotic microorganisms

2. The size of bacteria is:

- a) of the same order of magnitude as a human eukaryotic cell
- b) not of the same order of magnitude as a human eukaryotic cell
- c) measured in μm
- d) measured in mm

3. Regarding the relationship of bacteria to oxygen:

- a) aerobic bacteria do not require oxygen
- b) anaerobic bacteria require oxygen
- c) oxygen is toxic to anaerobic bacteria
- d) aerobic bacteria require oxygen

4. Which bacteria live permanently in the large intestine?

- a) fermentative bacteria
- b) *Escherichia coli*
- c) putrefactive bacteria
- d) non-pathogenic bacteria

5. Bacteria can be found:

- a) on the surface of organisms
- b) in the blood of a healthy person
- c) inside bacteriophages
- d) in the human large intestine

6. Bacteria cause:

- a) rabies
- b) tuberculosis
- c) chickenpox
- d) tetanus

7. Microscopic fungi are:

- a) prokaryotic organisms
- b) heterotrophic organisms
- c) capable of photosynthesis
- d) eukaryotic organisms

8. The first antibiotic was:

- a) isolated from a virus
- b) penicillin
- c) produced by the species *Penicillium notatum*
- d) vancomycin

9. A bacterial spore differs from the vegetative (mother) cell by:

- a) a different water content
- b) susceptibility to environmental factors
- c) metabolic activity
- d) taxonomic classification

10. The best-known bacterial agents of sexually transmitted diseases are:

- a) Salmonella
- b) *Mycobacterium tuberculosis*
- c) *Treponema pallidum*
- d) *Neisseria gonorrhoeae*

11. Causative agents of respiratory bacterial infections include representatives of the genera:

- a) Salmonella
- b) Mycobacterium (e.g., *M. tuberculosis*)
- c) Treponema
- d) Escherichia

12. Pathogenic bacteria can cause:

- a) salmonellosis
- b) cholera
- c) chickenpox
- d) measles

13. Agents of severe bacterial diarrheal diseases include genera:

- a) Salmonella
- b) Mycobacterium
- c) Shigella
- d) Escherichia

14. A plasmid:

- a) is something a bacterial cell can synthesize entirely on its own
- b) can be acquired by a bacterial cell from another cell
- c) is lost by the donor cell during conjugation
- d) is replicated during conjugation, so the donor cell does not lose it

15. Traits that can be plasmid-encoded include:

- a) resistance to antibiotics, chemotherapeutics, and heavy metals
- b) replication of the bacterial chromosome
- c) production of antibiotics and toxins
- d) degradation/oxidation of otherwise inert or toxic organic compounds

16. Genotypic methods for tracking bacterial antibiotic resistance:

- a) detect resistance genes or mutations and usually predict phenotypic resistance
- b) can detect brand-new, previously unknown genetic determinants
- c) can predict resistance and identify new variants of known resistance genes
- d) do not detect resistance genes without phenotypic expression

17. Gram staining allows:

- a) staining of bacterial spores
- b) staining of dividing cells
- c) identification of facultative anaerobic bacteria
- d) identification of virus-infected cells

18. Traits that can be plasmid-encoded include:

- a) production of restriction and modification enzymes
- b) formation of a bacterial nuclear membrane
- c) capacity for symbiosis
- d) synthesis of membrane-bound organelles in bacteria

19. Transfer of a plasmid from cell to cell:

- a) is divided into horizontal and vertical
- b) horizontal transfer can occur between different species and genera
- c) vertical transfer can occur between different species and genera
- d) also occurs during amitosis (binary fission)

20. Horizontal plasmid transfer:

- a) occurs during amitosis from mother to daughter cells
- b) occurs only between bacteria of the same genus
- c) is responsible for interspecies and intergenus transfer of antibiotic resistance
- d) occurs via conjugation

21. Targets of antimicrobial agents include:

- a) the cell wall
- b) lysosomes
- c) endoplasmic reticulum
- d) replication and transcription

22. Disruption of the microbiome is associated with:

- a) chronic inflammation of the large intestine, with strong evidence
- b) autism spectrum disorder, with moderate evidence
- c) obesity, with moderate evidence
- d) autoimmune diseases, with moderate evidence

23. Entamoeba histolytica – minuta form:

- a) forms quadrinucleate cysts
- b) does not form cysts
- c) is highly pathogenic
- d) occurs as a trophozoite

24. Entamoeba histolytica:

- a) humans are most often infected alimentarily by mature quadrinucleate cysts
- b) is the only pathogenic amoeba living in the human digestive tract
- c) parasitizes in the small intestine
- d) parasitizes in the large intestine

25. Eubiosis is:

- a) the optimal proportional representation of microorganisms in a biocenosis
- b) overgrowth of pathogenic microorganisms in the human body
- c) a state in which the balance of the normal microflora is disturbed
- d) absence of some microorganisms that are part of the normal human microbiota

26. Giardia intestinalis:

- a) parasitizes in the duodenum and upper small intestine
- b) is a flagellate
- c) parasitizes in the human large intestine
- d) has a spherical shape

27. Giardiasis most commonly presents with:

- a) anemia due to iron deficiency
- b) malabsorption syndrome
- c) mucus and fat in the stool
- d) blood in the stool

28. Cavity-dwelling (luminal) parasitic protozoa include:

- a) *Toxoplasma gondii*
- b) *Entamoeba histolytica*
- c) *Trichomonas vaginalis*
- d) *Plasmodium malariae*

29. Pathogenicity:

- a) is the ability of a microorganism to cause disease in a host
- b) is the ability of a microorganism to cause disease in every host
- c) opportunistic pathogens cause disease only under specific conditions or in immunodeficiency
- d) is a property of all microorganisms

30. The causative agent(s) of tertian (“three-day”) malaria are:

- a) *Plasmodium falciparum*
- b) *Plasmodium vivax*
- c) *Plasmodium malariae*
- d) *Plasmodium ovale*

31. *Toxoplasma gondii* trophozoites occur in two forms:

- a) pseudocysts
- b) tachyzoites
- c) tissue cysts
- d) bradyzoites

32. Structures that can extend from the cell membrane of parasitic protozoa include:

- a) flagella
- b) cilia
- c) pseudopodia
- d) undulating membranes

33. Which statement describes the term “dysbiosis”?

- a) a state when the microbiota is in balance (eubiosis)
- b) disruption of the composition of the microbiota associated with disease risk
- c) presence of only obligate pathogenic microbes in the intestine
- d) complete absence of microorganisms in the human body

34. Which example best illustrates an opportunistic infection?

- a) cholera caused by infection with *Vibrio cholerae* from contaminated drinking water
- b) urinary tract infection caused by *E. coli* after catheter insertion
- c) typhoid fever after infection with *Salmonella typhi*
- d) measles after contact with an infected person

35. Which term denotes disruption of the intestinal microbiota composition?

- a) eubiosis
- b) pathogenicity
- c) dysbiosis
- d) commensalism

36. What is the difference between an obligate and an opportunistic pathogen?

- a) an obligate pathogen almost always causes disease
- b) an opportunistic pathogen causes disease mainly when immunity is weakened or in an atypical environment
- c) an obligate pathogen is always present in the intestine
- d) an opportunistic pathogen occurs exclusively in children

37. What does the term “population prevalence” mean?

- a) the degree of severity of an infection
- b) the ability of a microorganism to cause disease
- c) the proportion of individuals in a population infected with a particular parasite or pathogen
- d) an important epidemiological indicator explaining local differences in morbidity

38. What do the terms eubiosis and dysbiosis mean?

- a) eubiosis is the balanced composition of the microbiota
- b) dysbiosis is the disruption of microbiota balance associated with various diseases
- c) eubiosis is a state when the host has no microorganisms
- d) dysbiosis denotes the ability of a microorganism to cause disease

39. How can a commensal and a symbiont be characterized?

- a) a commensal lives on the surface or inside the host without harming it
- b) a symbiont is a microorganism that is always pathogenic
- c) a symbiont lives in a stable relationship beneficial for both sides
- d) a commensal is a microorganism that always causes disease

40. How can cocci be arranged?

- a) as diplococci – pairs of spherical bacteria
- b) as vibrios – curved bacteria
- c) as bacilli – rod-shaped bacteria
- d) as streptococci – chains of spherical bacteria

41. Which of the following belong among spiral-shaped bacteria?

- a) vibrios – spiral-shaped cells
- b) spirilla – spiral-shaped cells
- c) staphylococci – spiral grape-like clusters
- d) bacilli – straight or curved spirals

42. Which bacteria require the presence of oxygen for their growth and metabolism?

- a) obligate aerobes
- b) obligate anaerobes
- c) facultative anaerobes
- d) aerotolerant anaerobes

43. Which bacteria can survive in the presence of oxygen but do not use it for metabolism?

- a) microaerophiles
- b) facultative anaerobes
- c) aerotolerant anaerobes
- d) obligate aerobes

44. Which option correctly matches the type of bacteria to its relationship to oxygen?

- a) obligate aerobe – growth only in the absence of oxygen
- b) obligate anaerobe – oxygen is toxic
- c) facultative anaerobe – growth with or without oxygen
- d) microaerophile – optimal growth at low oxygen concentration

45. Which statements about protozoa are correct?

- a) they are prokaryotic unicellular organisms
- b) they are eukaryotic and heterotrophic
- c) they never form cysts
- d) they can move by flagella, cilia, or pseudopodia

46. What methods are used in the diagnosis of protozoal infections?

- a) only cultivation on nutrient media
- b) microscopic examination of native or stained preparations
- c) immunological tests
- d) molecular biology methods

47. Which statements about *Trichomonas vaginalis* are correct?

- a) it is the largest human flagellate
- b) it causes radical vaginitis
- c) it has a pear-shaped body and 5 flagella
- d) it has an axial axostyle

48. Disruption of the microbiome is associated with:

- a) chronic inflammation of the large intestine
- b) clubfoot
- c) obesity
- d) autoimmune diseases

49. Where are bacteria commonly not found in a healthy person?

- a) in the skin
- b) in the intestine
- c) in the blood
- d) in the oral cavity

50. Which microorganism is the causative agent of tuberculosis?

- a) *Mycobacterium tuberculosis*
- b) *Neisseria gonorrhoeae*
- c) *Clostridium tetani*
- d) *Salmonella typhi*

51. Which bacterium is the causative agent of syphilis?

- a) *Shigella dysenteriae*
- b) *Treponema pallidum*
- c) *Escherichia coli*
- d) *Vibrio cholerae*

52. How does botulinum toxin act on the body?

- a) stimulates acetylcholine release at synapses
- b) blocks neuromuscular transmission and causes paralysis
- c) promotes division of nerve cells
- d) degrades phospholipids of the cell membrane

53. Which *Plasmodium* species can form hypnozoites and cause malaria relapses?

- a) *P. falciparum* and *P. malariae*
- b) *P. vivax* and *P. ovale*
- c) *P. falciparum* and *P. vivax*
- d) *P. malariae* and *P. ovale*

54. Which stages of *Toxoplasma gondii* are correctly matched to their role in infection?

- a) sporozoites – transmission by mosquito
- b) bradyzoites – chronic latent phase in tissue cysts
- c) hypnozoites – persistence in muscle cells
- d) tachyzoites – acute phase of infection

55. Which developmental stages match the listed parasite groups?

- a) hypnozoites – *Plasmodium vivax* and *Plasmodium ovale*
- b) sporozoites – infectious form of *Plasmodium* transmitted by mosquitoes
- c) tachyzoites – rapidly dividing forms of *Toxoplasma gondii*
- d) bradyzoites – latent forms of *Plasmodium falciparum*

56. Match the virus to a use or property:

- a) retroviruses – stable integration of foreign DNA into the host genome
- b) adenoviruses – transient gene expression with marked integration
- c) adeno-associated viruses – high safety and low immunogenicity
- d) rabies virus – commonly used in genetic engineering

57. Match the disease to the causative agent:

- a) tuberculosis – *Mycobacterium tuberculosis*
- b) tetanus – *Treponema pallidum*
- c) syphilis – *Treponema pallidum*
- d) gonorrhea – *Clostridium tetani*

58. Match the disease to the causative agent:

- a) salmonellosis – *Salmonella enterica*
- b) severe diarrheal diseases – *Neisseria gonorrhoeae*
- c) enteropathogenic infections – *Escherichia coli*
- d) botulism – *Shigella dysenteriae*

59. Match the disease to the causative agent:

- a) toxoplasmosis – *Toxoplasma gondii*
- b) malaria – *Clostridium botulinum*
- c) relapsing malaria – *Plasmodium vivax*
- d) blood cysts – tachyzoites of *Toxoplasma gondii*

60. Match the disease to the causative agent:

- a) HIV – acquired immunodeficiency syndrome (AIDS)
- b) rabies virus – respiratory infections
- c) adenoviruses – hepatitis B
- d) retrovirus HTLV-1 – T-cell leukemia

Answer Key to Model Questions

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| 1. FTFT | 21. TFFT | 41. FTFF |
| 2. FTTF | 22. TTTT | 42. TFFF |
| 3. FFTT | 23. TFFT | 43. FFTF |
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14 Viruses

Viruses are infectious acellular particles. For replication, they require a living host cell. A complete infectious viral particle is called a **virion** and consists of nucleic acid (DNA or RNA, but never both) enclosed in a protein coat, known as **the capsid**. The capsid is formed by capsomeres and carries surface proteins, which are ligands that ensure specific binding to receptors on the target cell. The capsid, together with the nucleic acid, is referred to as the nucleocapsid. According to capsid morphology, we distinguish an icosahedral (cubic) and a helical (spiral) type of nucleocapsid. Surface proteins constitute antigens recognized by the immune system. Some virions also contain internal “core” proteins, including enzymes necessary for replication (e.g., reverse transcriptase and integrase in retroviruses). A portion of viruses exit cells by budding and thereby acquire a lipid envelope from the host biomembrane, which facilitates fusion with cells and may hinder immune recognition.

The relationship between a virus and its host is strictly specific (**cellular tropism**) and is tied to the presence of the appropriate receptor (on the surface of the host cell). It also depends on the presence of a suitable receptor (susceptibility) and the ability of the virus to complete the entire replication cycle in the cell (**permissivity**). There is also an interspecies barrier that prevents most viruses from crossing between species. The occurrence of diseases depends on the virulence (degree of pathogenicity) of the agent and the conditions under which it is transmitted. **Obligate cellular biotrophy** means that viruses can replicate only inside living host cells. The ability of a given virus to infect only certain cells, tissues, or species is called **viral tropism**. Based on the extent of the affected population, we distinguish among local outbreaks, epidemics, and pandemics. Spread often occurs via droplets and direct contact, but transmission by vectors, such as mosquitoes, or by infected cells is also known to occur.

The **life cycle** of a virus includes adsorption to the cell, penetration, uncoating and release of the genome, replication of the nucleic acid, assembly and maturation of new virions, and their release by lysis or budding. Viruses generally do not contain genes for rRNA or tRNA; therefore, for their replication and synthesis of their proteins, they utilize the host’s protein-synthesizing apparatus, although some viruses may contain genes for ribosomal proteins that have the capacity to be incorporated into the host ribosome.

The **infectious cycle** of a virus can be **lytic** or **lysogenic**. In the **lytic cycle**, the virus immediately reprograms the cell to produce virions, which typically result in the lysis of the host cell. In the **lysogenic cycle**, the phage genome integrates into the host DNA and persists in it as a prophage. This state is maintained by the activation of a repressor that prevents the expression of genes involved in the lytic cycle. In retroviruses, after reverse transcription of the RNA genome into DNA, the viral DNA integrates into the host genome as a provirus and may later become transcriptionally active, leading to the production of new viral particles (also called reactivation into a lytic phase). Since viruses have minimal intrinsic metabolism, the host's specific immunity plays a fundamental role in controlling them. Antiviral agents are

targeted at selected steps of the viral life cycle (entry, replication, maturation), whereas antibiotics act against concomitant bacterial infections.

Viruses are classified according to multiple criteria. The most important are the type of genome (DNA or RNA, single-stranded or double-stranded) and capsid architecture (icosahedral or helical). By surface structure, they are divided into **enveloped viruses** (bearing a lipid envelope derived from host membranes) and **non-enveloped**, or “naked,” viruses (comprising a capsid only). Viral classification also reflects host range, tissue tropism, and genome properties. We distinguish bacterial viruses (**bacteriophages**), which infect prokaryotic cells, plant viruses (**phytotropic**), and animal viruses (**zoonotic**).

The **Baltimore classification** divides viruses into seven groups based on genome type and replication strategy. An overview with examples appears below.

Class	Genome type / polarity	Envelope	Site of replication	Key enzymes / notes	Example families/genera	Model virus
I	dsDNA	often yes/no	nucleus (poxviruses – cytoplasm)	host DNA polymerase; some encode their own enzymes	Herpesviridae, Adenoviridae, Poxviridae, Papillomaviridae, Polyomaviridae	HSV-1, Variola virus
II	ssDNA (+)	usually no	nucleus	formation of a dsDNA intermediate	Parvoviridae, Circoviridae	Parvovirus B19
III	dsRNA	often no	cytoplasm	viral RNA-dependent RNA polymerase	Reoviridae	Rotavirus
IV	(+) ssRNA	often yes	cytoplasm	genome functions as mRNA; synthesis of a (–)RNA template	Picornaviridae, Flaviviridae, Togaviridae, Coronaviridae	Poliovirus, SARS-CoV-2
V	(–) ssRNA	yes	cytoplasm (orthomyxoviruses – nucleus)	RdRp must be packaged in the virion	Orthomyxoviridae, Paramyxoviridae, Rhabdoviridae, Filoviridae	Influenza A virus, Rabies virus, Ebola virus
VI	ssRNA-RT (+)	yes	cytoplasm + nucleus	reverse transcriptase, integrase, protease; provirus stage	Retroviridae (Lentivirus)	HIV-1
VII	dsDNA-RT (partially ds)	yes	nucleus + an RT step	RNA intermediate and reverse transcription back to DNA	Hepadnaviridae	HBV (hepatitis B virus)

(RdRp = RNA-dependent RNA polymerase; ssDNA/ssRNA = single-stranded DNA/RNA; dsDNA/dsRNA = double-stranded DNA/RNA; “+” and “–” denote positive-sense (same polarity as mRNA) and negative-sense (complementary to mRNA), respectively; RT+ = viruses that encode reverse transcriptase (RT); RT– = viruses that do not encode reverse transcriptase)

Retroviruses may be exogenous (e.g., HIV) or endogenous (HERV), with endogenous retroviruses constituting ~8% of the human genome and influencing physiological processes (e.g., embryonic development). Retroviruses undergo recombination during genome packaging. The acquisition of foreign sequences can generate transforming variants capable of inducing rapid malignant transformation in infected cells. Reverse transcriptase has three activities: RNA-dependent DNA polymerase, DNA-dependent DNA polymerase, and RNase H.

The **HIV genome** consists of two identical copies of +ssRNA that carry three structural genes (gag, pol, and env) and six regulatory genes (tat, rev, nef, vif, vpr, and vpu; in HIV-2, also vpx). On the virion surface are glycoproteins that mediate cell entry: gp120 binds the host-cell receptor CD4 and the coreceptors (CCR5 or CXCR4), while gp41 mediates fusion of the viral and cellular membranes.

DNA viruses such as HPV, the polyomavirus SV40, and herpesviruses may have oncogenic potential because, for example, HPV encodes the oncoproteins E6 and E7 (E6 binds p53 and E7 binds RB1), which “disable the G1/S checkpoint” and thereby permit the synthesis of all proteins required for DNA replication – above all, the viral genome. If a cell survives the viral assault, replication of its chromosomal DNA begins without the repair of mutations, which can lead to malignant transformation. HPV has a small genome with overlapping genes, contains no enzymes within the virion, and replicates in the host cell nucleus. The oncogenic HPV types include the so-called high-risk serotypes HPV16 and HPV18, which are most frequently associated with cervical carcinoma. Other types, such as HPV11, are low-risk and cause warts. The **Epstein-Barr virus** (a herpesvirus) is associated with Burkitt lymphoma and nasopharyngeal carcinoma, while HHV-8 is associated with Kaposi sarcoma. The family Poxviridae comprises large DNA viruses that replicate in the cytoplasm. They cause smallpox (variola), which must be distinguished from chickenpox (varicella), caused by herpesviruses.

A distinct group of infectious agents is formed by subviral particles – viroids (small circular ssRNA without an envelope), satellite viruses (defective RNA that requires a helper virus), and prions (infectious proteins without nucleic acids).

Viral evolution is driven by mutations and recombination. A high mutation rate is typical, especially among ssRNA viruses, because their polymerases lack proofreading capabilities, resulting in antigenic drift. These continuous minor variations of surface antigens reduce the effectiveness of immunity and contribute to the development of epidemics. In viruses with segmented genomes, simultaneous infection of a single cell by two strains can lead to the exchange (reassortment) of certain segments, resulting in the emergence of antigenic shift – a fundamental change in properties and antigens with pandemic potential.

Genome type/class	Agent (genus / family)	Disease
dsDNA / I	HSV-1, <i>Herpesviridae</i>	Herpes labialis
	Varicella-zoster virus, <i>Herpesviridae</i>	Chickenpox / shingles
	Variola virus, <i>Poxviridae</i>	Smallpox
	Papillomaviruses, <i>Papillomaviridae</i>	HPV infections
	Adenoviruses, <i>Adenoviridae</i>	Adenoviral infections
ssDNA / II	Parvovirus B19, <i>Parvoviridae</i>	Parvoviral infection
dsRNA / III	Rotavirus, <i>Reoviridae</i>	Pediatric gastroenteritis
(+)ssRNA / IV	Poliovirus, <i>Picornaviridae</i>	Poliomyelitis
	HAV, <i>Picornaviridae</i>	Hepatitis A
	Flaviviruses, <i>Flaviviridae</i>	Dengue, Zika, Yellow fever
	SARS-CoV-2, <i>Coronaviridae</i>	COVID-19
(–)ssRNA / V	Influenza virus A/B, <i>Orthomyxoviridae</i>	Influenza
	Measles virus, <i>Paramyxoviridae</i>	Measles
	Mumps virus, <i>Paramyxoviridae</i>	Mumps
	Respiratory syncytial virus, <i>Pneumoviridae</i>	RSV infections
	Rabies virus, <i>Rhabdoviridae</i>	Rabies
	Filoviruses, <i>Filoviridae</i>	Ebola / Marburg
ssRNA – RT / VI	HIV-1/2, <i>Retroviridae</i>	AIDS
dsDNA – RT / VII	HBV, <i>Hepadnaviridae</i>	Hepatitis B



Model questions

1. Oncogenic viruses:

- a) can cause cancer
- b) can eliminate cancer
- c) include Epstein-Barr virus, hepatitis B virus, and human papillomavirus
- d) include HTLV (human T-cell lymphotropic virus), hepatitis C virus, and Kaposi's sarcoma-associated herpesvirus

2. A phage is a virus associated with a:

- a) eukaryotic cell
- b) prokaryotic cell
- c) plant cell
- d) animal cell

3. High-risk viruses for cervical carcinoma are:

- a) HPV11
- b) HPV14
- c) HPV16
- d) HPV18

4. For cell entry, HIV does not require:

- a) gp120
- b) gp41
- c) gp141
- d) gp18

5. Integration of a phage genome causes:

- a) activation of early viral genes
- b) activation of late viral genes
- c) activation of a repressor
- d) activation of a corepressor

6. Based on the presence of an envelope, viruses are classified as:

- a) enveloped
- b) semi-enveloped
- c) naked
- d) stripped

7. Poxviruses cause:

- a) rabies
- b) chickenpox
- c) smallpox
- d) encephalitis

8. Viruses are classified by:

- a) genome
- b) capsid
- c) staining
- d) surface projections

9. Acellular (non-cellular) human pathogens include:

- a) yeasts
- b) prions
- c) viruses
- d) bacteria

10. Viruses are:

- a) acellular pathogens of humans
- b) cellular pathogens of humans
- c) eukaryotic microorganisms
- d) prokaryotic microorganisms

11. Oncogenic viruses can cause:

- a) uncontrolled cell proliferation
- b) tuberculosis
- c) infectious jaundice and cholera
- d) the development of malignant tumors

12. Viruses may be composed of:

- a) proteins, nucleic acids, and lipids
- b) carbohydrates and proteins
- c) nucleic acids and proteins
- d) nucleic acids and lipids

13. Viruses replicate:

- a) only inside living cells
- b) by mitosis
- c) by amitosis
- d) only in cells of multicellular organisms

14. In humans, viruses cause:

- a) tuberculosis
- b) infectious hepatitis (“jaundice”)
- c) mosaic disease
- d) influenza

15. Infection of a cell by a virus proceeds through the phases:

- a) attachment, entry into the cell, and replication
- b) entry into the endoplasmic reticulum and replication
- c) prophase, metaphase, and anaphase
- d) S phase, G2 phase, and prophase

16. Where do viruses replicate?

- a) inside infected cells
- b) in red blood cells
- c) inside host cells
- d) in nutrient solutions

17. A bacteriophage is:

- a) a type of bacterium
- b) a type of white blood cell
- c) a virus that infects bacteria
- d) a bacterium that engulfs other bacteria

18. The protein coat of a virus is called a:

- a) capsid
- b) spore
- c) murein
- d) peptidoglycan

19. What may be present on the surface of the protein coat of larger viruses?

- a) a lipid envelope
- b) a capsomere
- c) mRNA
- d) peptidoglycan

20. Regarding AIDS, it is true that:

- a) the virus is transmitted through blood and can be a risk with frequent transfusions
- b) risk groups are men who have sex with men, people with risky sexual behavior, and people who share injection needles
- c) death is often due to opportunistic infections caused by parasites, fungi, yeasts, and some viruses
- d) it occurs only in Africa and Asia

21. Diseases of animals or humans caused by viruses include:

- a) influenza
- b) poliomyelitis
- c) AIDS
- d) measles

22. By host type, viruses are divided into:

- a) icosahedral and helical
- b) bacterial, plant, and animal viruses
- c) enteric, respiratory, and arboviruses
- d) bacteriophages, phytotropic, and zoonotic viruses

23. Viral reproduction:

- a) is independent of the host cell
- b) occurs on the surface of the host cell
- c) involves the formation of new virions
- d) is termed amitosis

24. True statements about viruses:

- a) they are prokaryotic cells
- b) virus particles are called virions
- c) they have no genetic information
- d) their diseases are treated with antibiotics

25. Which of the following is true of viruses?

- a) they are intracellular parasites of other organisms
- b) they are also classified by the kinds of cells they parasitize
- c) they are also classified by the type of nucleic acid
- d) they are divided into those parasitizing plants, animals, and bacteria

26. What basic morphological types of capsid are distinguished in viruses?

- a) icosahedral
- b) helical
- c) cubic-helical
- d) filamentous

27. Which enzymes belong among the “core proteins” of some virions?

- a) reverse transcriptase
- b) integrase
- c) ribosomes
- d) host cell DNA polymerase

28. What does the term “cellular tropism” of a virus mean?

- a) the ability to infect any cells
- b) the specificity of a virus toward certain types of host cells
- c) the ability to form a capsid
- d) general resistance to the host’s immune system

29. What does the term “permissiveness” of a host cell denote?

- a) the presence of a suitable receptor
- b) the ability of the cell to allow completion of the entire viral replication cycle
- c) susceptibility of the cell to apoptosis
- d) genetic stability of the virus

30. What does the term “virulence” of a virus express?

- a) the degree of pathogenicity and ability to cause disease
- b) the presence of a capsid
- c) the ability to infect any organism
- d) the mode of transmission

31. What does the term “obligate cellular biotrophy” mean?

- a) the virus can replicate only inside living host cells
- b) the virus can replicate in all cells
- c) the virus infects only bacteria
- d) it is a synonym for pandemic spread

32. What is the correct order of phases in the viral life cycle?

- a) adsorption, penetration, uncoating of the genome, replication, assembly, release
- b) penetration, adsorption, uncoating of the genome, assembly, replication, release
- c) uncoating, adsorption, assembly, replication, release
- d) adsorption, uncoating, penetration, replication, release, assembly

33. Which genes are usually absent in most viruses?

- a) genes for rRNA
- b) genes for tRNA
- c) genes for structural proteins of the capsid
- d) genes for regulatory proteins specific to the virus

34. Viruses are classified according to:

- a) the type of genome (DNA or RNA)
- b) the capsid structure (icosahedral, helical)
- c) the mode of division of the cell they infect
- d) the number of mitochondria in the host cell

35. Which statements correctly characterize enveloped viruses?

- a) they have a lipid envelope derived from the host membrane
- b) they are always more resistant to disinfection than naked viruses
- c) the envelope facilitates fusion with cell membranes
- d) they do not contain a capsid, only nucleic acid

36. Which statements about retroviruses are correct?

- a) retroviruses can be exogenous or endogenous, with endogenous retroviruses forming about 8% of the human genome
- b) the reverse transcriptase of retroviruses has only one activity – RNA-dependent DNA polymerase activity
- c) retroviruses undergo recombination during genome packaging, which can lead to the emergence of transforming variants
- d) retroviruses contain DNA as their primary genome, which is immediately transcribed in the nucleus

37. Which statements about HIV are correct?

- a) the genome of HIV consists of two identical copies of +ssRNA and, in addition to structural genes, also contains regulatory genes such as tat, rev, nef, and vif
- b) HIV uses glycoprotein gp120 to bind to the CD4 receptor and to the coreceptors CCR5 or CXCR4
- c) gp41 mediates binding to CD8+ T lymphocytes and their apoptosis
- d) HIV-2 additionally contains the vpx gene, which does not participate in human cell infection

38. Viruses are divided according to host range into:

- a) bacterial viruses (bacteriophages)
- b) plant viruses (phytotropic)
- c) animal viruses (zoonotic)
- d) viruses of the endoplasmic reticulum

39. Which statements correctly compare retroviruses and DNA viruses?

- a) retroviruses carry an RNA genome and need reverse transcriptase, whereas DNA viruses carry a DNA genome and use either the host's DNA polymerases or their own
- b) the oncogenic potential of retroviruses is linked to their ability to integrate into the host genome, while the oncogenic potential of DNA viruses (e.g., HPV) is mediated by viral oncoproteins
- c) retroviruses have enzymes already incorporated in the virion, DNA viruses never contain them
- d) DNA viruses such as HPV and herpesviruses replicate exclusively in the cytoplasm, whereas retroviruses replicate in the nucleus

40. Baltimore classification of viruses is based on:

- a) the type of nucleic acid of the virus (DNA/RNA, single-stranded/double-stranded)
- b) the mechanism of viral mRNA synthesis
- c) the shape of the capsid (icosahedral vs. helical)
- d) the presence or absence of a lipid envelope

41. Which statement about the lytic cycle is correct?

- a) the viral genome integrates into the host DNA
- b) immediate production of virions and lysis of the cell occurs
- c) the virus persists as a prophage
- d) the host cell remains viable

42. Why can't viruses synthesize their proteins independently of the host?

- a) they lack a capsid
- b) they lack ribosomes, rRNA, and tRNA
- c) they lack a lipid envelope
- d) their genome is too small

43. What factors determine the specificity of the virus-host relationship?

- a) presence of an appropriate receptor on the cell surface (susceptibility)
- b) the cell's ability to complete the replication cycle (permissivity)
- c) capsid type (icosahedral or helical)
- d) virulence of the particular virus

44. What is antigenic shift?

- a) a minor cumulative change in antigens over time
- b) a major change in properties and antigens due to reassortment
- c) most common in viruses with segmented genomes
- d) it leads to higher efficacy of antivirals

45. Which statement about retroviral recombination is correct?

- a) it occurs during genome packaging
- b) it does not change the viral genome
- c) it can lead to the emergence of transforming variants
- d) it has no relevance to oncogenic potential

46. Which statements about HPV are true?

- a) it lacks enzymes within the virion
- b) replication occurs in the host cell nucleus
- c) HPV16 and HPV18 are high-risk types linked to cervical carcinoma
- d) HPV11 is a low-risk type causing warts

47. Epstein-Barr virus (EBV) is associated with:

- a) Kaposi's sarcoma
- b) nasopharyngeal carcinoma
- c) Burkitt lymphoma
- d) colorectal cancer

48. What causes smallpox (variola)?

- a) herpesviruses
- b) poxviruses
- c) retroviruses
- d) adenoviruses

49. Chickenpox (varicella) is caused by:

- a) variola virus
- b) herpesviruses
- c) retroviruses
- d) HPV

50. What are the properties of viroids?

- a) small circular ssRNA without a coat
- b) infectious proteins without nucleic acids
- c) they require a helper virus for replication
- d) they cause plant diseases

51. What characterizes prions?

- a) small RNA molecules without a coat
- b) infectious proteins without nucleic acids
- c) they can cross species
- d) they require a helper virus for replication

52. What is reassortment in viruses with segmented genomes?

- a) recombination within a single RNA molecule
- b) exchange of entire segments between two viruses infecting the same cell
- c) the process responsible for antigenic drift
- d) a process that leads to antigenic shift

53. Which mechanisms drive viral evolution?

- a) mutations
- b) recombination
- c) reassortment
- d) horizontal gene transfer between hosts

54. Which statements correctly describe antigenic drift vs. antigenic shift?

- a) drift consists of small, gradual antigenic changes
- b) shift consists of major antigenic changes
- c) drift is caused by mutations due to polymerases lacking proofreading
- d) shift results from reassortment of a segmented genome

55. Why do ssRNA viruses have high mutation rates?

- a) their polymerases lack proofreading
- b) they have segmented genomes
- c) they contain many regulatory genes
- d) their replication occurs in the host nucleus

56. What is typical of the lytic cycle?

- a) the viral genome integrates into host DNA
- b) immediate synthesis of virions
- c) infection ends with lysis of the host cell
- d) long-term latent persistence of the virus

57. What characterizes the lysogenic cycle of bacteriophages?

- a) the viral genome integrates into the host DNA as a prophage
- b) replication of virions begins immediately after infection
- c) activation of a repressor prevents expression of lytic-cycle genes
- d) the virus replicates exclusively in the cytoplasm

58. What can trigger a switch from the lysogenic to the lytic cycle?

- a) activation of the repressor
- b) inactivation of the repressor due to stress (UV radiation, chemicals)
- c) spontaneous integration of DNA into the host chromosome
- d) high virulence of the virus

59. Which statement correctly describes the difference between lytic and lysogenic cycles?

- a) the lytic cycle leads to the destruction of the host cell, the lysogenic cycle does not
- b) in the lysogenic cycle, the virus immediately produces new particles
- c) the lytic cycle involves integration of the viral genome into host DNA
- d) the lysogenic cycle allows the virus to persist latently in the host

60. Which statements about SARS-CoV-2 (the cause of COVID-19) are correct?

- a) it is an RNA virus with a positive-sense single-stranded genome (+ssRNA)
- b) it belongs to the family Coronaviridae, genus Betacoronavirus
- c) the surface “spike” glycoprotein mediates binding to the host ACE2 receptor
- d) it contains reverse transcriptase and integrates into host DNA

Answer Key to Model Questions

- | | | |
|----------|----------|----------|
| 1. TFTT | 21. TTTT | 41. FTFF |
| 2. FTFF | 22. FTFT | 42. FTFF |
| 3. FFTT | 23. FFTF | 43. TTFT |
| 4. FFTT | 24. FTFF | 44. FTTF |
| 5. FFTF | 25. TTTT | 45. TFTF |
| 6. TFTF | 26. TTFF | 46. TTTT |
| 7. FFTF | 27. TTFF | 47. FTTF |
| 8. TTFF | 28. FTFF | 48. FTFF |
| 9. FTTF | 29. FTFF | 49. FTFF |
| 10. TFFF | 30. TFFF | 50. TTFT |
| 11. TTFT | 31. TFFF | 51. FTTF |
| 12. TFTF | 32. TFFF | 52. FTFT |
| 13. TFFF | 33. TTFF | 53. TTTF |
| 14. FTFT | 34. TTFF | 54. TTTT |
| 15. TFFF | 35. TFTF | 55. TFFF |
| 16. TFTF | 36. TFTF | 56. FTTF |
| 17. FFTF | 37. TTFF | 57. TFTF |
| 18. TFFF | 38. TTTF | 58. FTFF |
| 19. TFFF | 39. TTFF | 59. TTFT |
| 20. TTTF | 40. TTFF | 60. TTTF |

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