

SUBJECT:	<b>CELL BIOLOGY</b>		
CREDITS:	Total: <input type="text" value="7"/>	Theory: <input type="text" value="4"/>	Practical: <input type="text" value="3"/>

## GENERAL OBJECTIVE

This subject aims to provide students with a basic conceptual and practical understanding of cell structure and cell functions that will allow them to understand the biological basis of pathology and therapeutics.

## SPECIFIC OBJECTIVES

- To be able to identify the different cell organelles.
- To be able to establish relationships between intracellular structures and the functions that they carry out.
- To recognize the relationships that exist between the functions of the different intracellular organelles.

## PROGRAMME

### Theory

#### Introduction

- 1. The cell as a structural and functional unit of living systems.** General characteristics of the eukaryotic cell and differences with the prokaryotic cell. Concepts of functional compartmentalization in eukaryotic cells.
- 2. Prebiotic evolution.** Oparin's Theory. Abiotic synthesis of organic compounds on early Earth. Importance of the formation of the first cell membrane. Formation of the first cells.
- 3. Cell development.** From the first prokaryotes to photosynthesizing prokaryotes, the food crisis. The aerobic crisis, appearance of aerobic cells. The transition from prokaryotes to eukaryotes and the endosymbiotic theory. Transition from unicellular to multicellular life.

#### Experimental methods for studying cells

- 4. Microscopic techniques.** Basic principles of microscopy and preparation of material. Optical microscopy, different types of microscope. Confocal microscopy. Electronic microscopy: transmission and scanning. Cryo-fracture.
- 5. Fractionation techniques and cell cultures.** Cell fractionation techniques, homogenization and different types of centrifugation. Basic cell culture techniques: primary cultures and established cell lines. Microinjection.
- 6. Techniques for the localization and quantification of molecules.** Cytochemical and immunocytochemical techniques. Western blotting. Radioactive carriers and autoradiography. In situ hybridization.

#### Intracellular compartments: intracellular distribution of molecules and maintenance of cell structure

- 7. Structure of cell membranes. Composition and functions.** Structural organization of the different components of the membrane: fluid mosaic model. The plasma membrane. Permeability of cell membranes. Ultra-structural analysis.

**8. Compartmentalization.** Concept of the eukaryotic cell compartment. Topological relationships between the different intracellular compartments: vesicular transport. Protein traffic, molecular signaling mechanisms. Concept of *functional polarity*.

**9. Molecular and structural characteristics of the endoplasmic reticulum.** Structure and intracellular localization of the smooth and rough endoplasmic reticulum. Functions of the smooth endoplasmic reticulum, phospholipid synthesis. Relationship between the smooth and rough reticulum.

**10. The biosynthetic machinery of the rough endoplasmic reticulum.** Protein biosynthesis in the rough endoplasmic reticulum. Protein and lipid modifications. Classification and distribution of resident and secretion proteins.

**11. The Golgi apparatus.** Structural organization of the Golgi apparatus. Functional and structural dissection of the Golgi apparatus. The *cis*-Golgi distribution network and the transreticular region (TGN). Intra-Golgi localization of posttranslational protein processing.

**12. Exocytosis.** Formation of secretory vesicles in the *trans*-Golgi network. Protein secretion, molecular classification and intracellular distribution mechanisms. Constitutive secretion and regulated secretion.

**13. Lysosomes.** Structure and function. Structural heterogeneity of lysosomes. Origin of lysosomes. Molecular mechanisms for the transport of lysosomal enzymes. The mannose receptor. Lysosomal disorders.

**14. Endocytosis.** Concepts of *phagocytosis*, *pinocytosis*, *potocytosis* and *receptor-mediated endocytosis*. Structure and function of the endocytic compartment, endosomes. Transcytosis in epithelial cells. Endocytosis of viruses and toxins.

**15. Molecular mechanisms of vesicular transport.** Protein classification mechanisms in the different intracellular compartments. Structures and molecules involved in intracellular transport. The structure of coatomer. Fusion and twinning of vesicles.

**16. Mitochondria and peroxisomes.** Structural and functional compartmentalization of mitochondria. Mitochondrial protein and lipid transport. Mitochondrial division. Peroxisomes: structure, function and biosynthesis. Peroxisomal disorders.

**17. The cytosol.** Concept. Principal components of the cytosol. Ribosomes: composition and structure. Polysomes and protein synthesis on cytosolic ribosomes. The proteasome: mechanisms of cytosolic protein degradation.

## Cell shape and motility: the cytoskeleton

**18. The cytoskeleton** Concept of the cytoskeleton. Principal types of filament that make up the cytoskeleton: intermediate filaments, tubulin filaments and actin filaments. Principal functions of the cytoskeleton.

**19. Intermediate filaments.** The intermediate filaments. Composition of intermediate filaments. Types, distribution and cellular localization. Functions of the different types of intermediate filament.

**20. Microtubules and movement associated with microtubules.** Composition and structure of microtubules. Microtubule organizing centres. Microtubule-associated proteins (MAP). Motor proteins. Microtubule-mediated intracellular transport. Structure of cilia and flagella. Mechanisms of ciliary movement.

**21. Actin filaments and cell motility.** Components and organization of actin filaments. Actin-binding proteins. Different models of actin filament organization. Contractile structures in nonmuscular cells. Intracellular transport and actin filaments.

## Relationship between the cell and its environment

**22. Cell junctions.** Functional types of cell junctions. Occluding or hermetic junctions. Actin filament anchorage junctions: cell-cell (adhesive bands) and cell-matrix (focal contacts) adherens junctions. Intermediate filament anchorage junctions: desmosomes and hemidesmosomes. Communicating junctions.

**23. Intercellular adhesion.** Recognition and intercellular adhesion. Types of cell adhesion molecules. Function and cellular distribution. Regulation of expression during cell differentiation and embryogenesis.

**24. The extracellular matrix.** Organization and principal components. Structure and functional properties of principal components: collagen fibres, elastic fibres, fibronectin, hyaluronic acid and proteoglycans. Interactions of the extracellular matrix with the plasma membrane and the cytoskeleton: integrins.

## Maintenance, expression and replication of genetic information: the cell nucleus

**25. General characteristics of the cell nucleus.** General characteristics of the nucleus: size, shape and number of nuclei per cell. Main functions of the nucleus. General structure of the interphase nucleus. Nucleoli.

Chromocenters. Nucleoplasm. Nuclear envelope. Nuclear lamina. Nuclear matrix. Organization of DNA within the nucleus: chromosomes.

**26. Transport between the nucleus and the cytoplasm.** Structure and composition of nuclear pores. Mechanisms for transport of proteins from the cytoplasm to the nucleus. Mechanisms for transport of particles from the nucleus to the cytoplasm. Ion transport.

**27. The eukaryotic genome.** Concept of *genome*. Chromosomes as structures containing genetic material. Number and type of chromosomes. Genes within chromosomes. Principal chromosome regions: centromeres, telomeres and the origins of replication. Molecular composition of chromosomes.

**28. Chromosome organization.** Organization of interphase chromosome: the nucleosome and the 30 nm fibre. Levels of chromatin packaging. The metaphase chromosome. Chromatin condensation and transcriptional control: euchromatin and heterochromatin.

**29. Ribosome biogenesis: the nucleolus.** Ribosomal genes and nucleolar organizers. Synthesis and maturation of different ribosomal RNAs. The different structures of the nucleolus and their relationship with ribosome biogenesis. Changes in the nucleolus during the cell cycle.

**30. Chromatin replication.** Origins and units of replication. Chromatin condensation and replication. Organization of replication machinery, replisomes. Association of replication machinery and the nuclear matrix.

**31. The mechanism of the replication process.** Proteins involved DNA replication. Mechanism of DNA polymerase action. The beginning of DNA replication: recognizing origins and origin firing. The progression of replication. The end of replication.

## The growth and division of cells: the cell cycle

**32. The cell cycle.** Concept of *cell cycle*. The quiescent state or  $G_0$ . Phases of the cell cycle:  $G_1$ , S,  $G_2$  and M. Duration and principal features of each phase. Extracellular factors that regulate the cell cycle: growth factors, anchorage dependence and contact inhibition.

**33. Regulation of the cell cycle.** Identification of the cell cycle regulator molecules: cell fusion experiments, microinjection into the cytoplasm of *Xenopus* oocytes and yeast genetics. Cyclin-dependent kinases (CDKs) and the regulation of their activity. Specific CDK/cyclin complexes in the different phases of the cell cycle.

**34. The  $G_1$  phase.** The machinery of the cell cycle during quiescence. General signals for growth factors and anchorage. General signals for contact inhibition. Role of CDK4 and CDK2 in the progression of  $G_1$ . Phosphorylation of Rb during  $G_1$ .

**35. The S and  $G_2$  phases.** Induction of the S-phase: role of CDK2/cyclin E in the firing process. Progression of the S-phase: role of CDK2/cyclin A complexes.  $G_2$  as a holding phase. Regulation of entrance into mitosis.

**36. Mitosis.** Phases of mitosis: prophase, prometaphase, metaphase, anaphase, telophase and cytokinesis. Structure and function of the mitotic apparatus. Principal features of each phase of mitosis. Regulation of progression and exit from mitosis: role of CDK2/cyclin B complexes.

**37. Meiosis.** General properties of meiosis. The double meiotic cycle and its relationship with gametogenesis. Prophase of the first cycle, pairing and chromosomal exchanges. First division. Second division.

**38. Vigilance of the cell cycle (checkpoints).** Controls during  $G_1$ : control of growth and DNA damage. Controls in the S-phase: control of re-replication. Controls during  $G_2$ : relationship between the end of the S-phase and the beginning of mitosis and control of DNA damage. Control of mitosis: formation of mitotic spindle and equatorial plaque.

**39. Anomalies of cell proliferation: cancer.** Properties of cancerous cells. Oncogenes and tumour suppressor genes. Anomalies in the machinery of regulation of the cell cycle as essential elements in tumorigenesis. Alterations to checkpoints and oncogenesis.

**40. Cell death.** Physiological importance of cell death. Types of cell death: apoptosis and necrosis. General characteristics of apoptosis. Molecular mechanisms of apoptosis. Cell death during development. Genetic damage and apoptosis. Apoptosis and the cell cycle. Programme of practical training.

## Practical training

1. Preparation of samples for optical microscopy.
2. Components of the optical microscope Observation of cells from all five kingdoms.
3. Cell fractionation.
4. Electrophoresis and protein transfer.

5. Immunotransfer. Immunohistochemical and histochemical techniques.
6. Cell cultures. Cell cycle: methods for the detection of DNA synthesis. Autoradiography techniques.

Practical courses 1, 2 and 3 will last 2.5 hours and modules 4, 5 and 6 will last 5 hours.

**SESSIONS ON CELL ULTRASTRUCTURE:** observation and discussion of microscope slides that will be distributed to the groups for practical courses at the beginning of the course. Work will be done in students' own time.