Essentials of Cytology

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ESSENTIALS OF CYTOLOGY

PREFACE

Essentials of Cytology is an introductory text on cell structure and function designed specifically for undergraduate students. The study of the cell has today assumed an important part of subjects like Botany, Zoology, Microbiology and Biochemistry. A knowledge of the structure and function of the cell is also required for the applied biological sciences like medicine, agriculture and veterinary science.

The present book attempts to give an integrated account of the structure of the cell, both at microscopic and molecular levels, and its physiology. The layout of the chapters and the choice of topics have been designed to meet the needs of undergraduate students of Indian universities Students interested in greater detail are advised to study the companion volume, *Cell Biology*, by the same author.

Nagpur, July, 1983 C. B. POWAR

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1

INTRODUCTION

I. GENERAL ACCOUNT OF THE CELL

The first compound microscope was built by Robert Hooke, who used the term 'cell' in 1665 to describe the hollow spaces bound by cork in thin slices of cork. Thus started a new branch of biology—the study of the cell (*cytology*). In 1838 the German worker M. J. Schleiden discovered that all tissues of plants are made up of cells. In the same year, another German worker T. S. Schwann arrived at the same conclusion for animals. This concept was then applied to all living organisms. The findings of Schleiden and Schwann that all living organisms consist of cells are now referred to as the *cell theory* or the *cell principle*. The statements af Schleiden and Schwann have been slightly modified, and it is now held that living organisms consist of cells and cell products.

In the present section only a brief outline of the structure cell will be given. This will serve as an introduction to the organelles present in a generalized cell. The details of the structure and function of the organelles will be taken up in subsequent chapters.

Shape. The shape of the cell may be *variable*, *i.e.* constantly changing(e.g. *Amoeba* and leucocytes) or *fixed*. In the latter case the cell may be: (i) *flattened*, e.g, squamous epethelium, endothelium and the upper layers of the epidermis: (ii) *cuboidal*, e.g. in thyroid gland follicles; (iii) *columnar*, e.g. the cells lining the intestine; (iv) *discoidal* e.g. erythrocytes, (v) *spherical*, e.g. the eggs of many animals; (vi) *spindle-shaped*, e.g. smooth muscle fibres; (vii) *elongated*, e.g. nerve cells, or (viii) branched, e.g. pigment cells of the skin.

Size. The size of cells vary from the very small cells of bacteria (0.2 to 5.0 μ) to the very large egg of the ostrich (6″). In the latter a considerable part of the volume is made up of yolk, which is not protoplasm. Some nerve cells have axons as much as a metre in length.

The factors governing the size of the cell are: (i) the *nucleo-cytoplasmic ratio*, or the ratio between the volume of the nucleus and

the cytoplasm, (ii) the ratio of the cell surface to the cell volume, and (iii) the rate of metabolism.

Protoplasm and deutoplasm. The living substance of which the cell is made is called protoplasm. Protoplasm is differentiated into two regions, *nucleoplasm* and *cytoplasm*. *Nucleoplasm* is the protoplasm of the nucleus and cytoplasm the extra-nuclear protoplasm. The protoplasm of the cell contains many non-living substances which are mostly formed by the cell. These substances are collectively called *deutoplasm*, and include yolk bodies, lipid droplets, secretory granules and pigment. The cytoplasm may be differentiated into a granular peripheral region called *ectoplasm* (*plasmagel*, *cortex*) and a granular central region called *endoplasm* (*medulla*).

The cytoplasmic structures include the plasma membrane, the endoplasmic reticulum, the ribosomes, the Golgi complex, the mitochondria, the chloroplasts (in plant cells), the centrioles, the lysosomes, the cilia, the flagella and the vacuoles. The nucleoplasm consists of the nuclear envelope, nuclear sap, chromatin, chromosomes and nucleoli (Fig.1.1).

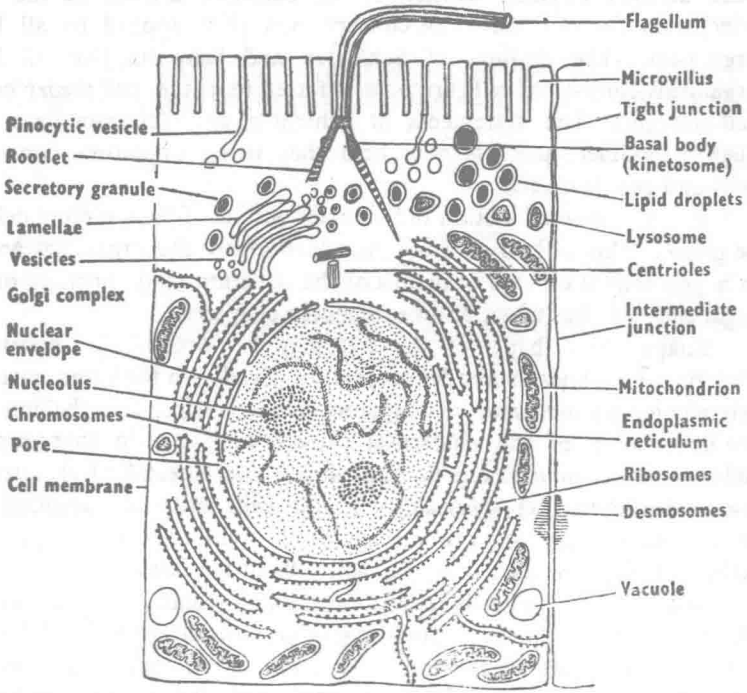

Fig. 1.1. Generalized diagram of an animal cell.

Plasma membrane. The cell is bounded by a lipoprotein membrane which is selectively permeable, and in specialized cells like nerve cells

is responsible for the conduction of impulses (excitation). The plasma membrane together with the surrounding cell cement forms the *cell membrane*, the structure visible under the light microscope. In plant cells the cell membrane is surrounded by a *cell wall.*

The **Golgi complex** consists of a system of smooth membranes in the form of cisternae and vesicles. Various functions have been ascribed to the Golgi complex. The main function is the concentration and budding off of secretory products and carbohydrate synthesis.

Scattered through the cytoplasm are granular or rod-like bodies called **mitochondria**. They contain the enzymes for the Krebs cycle and oxidative phosphorylation. Mitochondria have been called the powerhouses of the cell and are the centres of cell respiration and release of energy.

Chloroplasts are chlorophyll-containing bodies found in plant cells. They contain structures called *quantosomes* which are the units of photosynthesis.

Lysosomes are particles consisting of hydrolytic enzymes enclosed within lipoprotein membranes. The lysosomes have been called "suicide bags" because the enzymes cause breakdown and death of the cell. Lysosomes are concerned with extra-cellular and intra-cellular digestion, cell breakdown, penetration of the sperm, and initiation of cell division.

The cytoplasm contains **vacuoles**, which are fluid-filled spaces enclosed by membranes. They are well developed in plant cells, but absent from most animal cells, except the Protozoa.

In both plants and animals some cells have hair-like structures called **cilia** and **flagella**. At the base of each cilium and flagellum is a *basal body* or *kinetosome*.

Eukaryote cells have **nuclei** which are bounded by a double membraned *nuclear envelope* perforated by *pores*. The nucleus is filled with a colourless fluid called *nuclear sap*. Within the sap is a network of *chromatin*. The nucleus may contain one of more *nucleoli* which are the storage sites of nuclear RNA. During cell division filamentous bodies called *chromosomes* appear in the nucleus. The chromosomes contain DNA which acts as a template for the synthesis of RNA.

II. PROKARYOTE AND EUKARYOTE CELLS

Organisms in which the nuclear material is not bounded by a definite nuclear membrane are called *prokaryotes*, e.g bacteria and blue-green algae (now included in bacteria). Organisms in which the nucleus has a definite nuclear membrane are known as *eukaryotes*, e.g. all other plants and animals. The cells of prokaryotes and eukaryotes differ fundamentally in many ways.

1. **Nuclear membrane.** As mentioned previously *prokaryotic cells* lack a nuclear membrane while *eukaryotic cells* have a definite nuclear membrane.

2. **Chromosomes.** In *prokaryotes* the genetic meterial consists of nucleic acid (DNA). The DNA molecule is circular and lies in a tangled mass *(nucleoid)*. In *eukaryotes* the nucleic acid (DNA) is associated with proteins to form definite bodies called *chromosomes.*

3. **Cytoplasmic organelles.** Prokaryotic cells have no endoplasmic reticulum, Golgi complex, mitochondria, lysosomes or centrioles. The enzymatic functions of the mitochondria are carried out by the cell membrane which is folded inwards at various points. *Eukaryotic* cells have definite internal membranous structures like the endoplasmic reticulum, Golgi complex, mitochondria and lysosomes.

4. **Cell wall.** The cell wall of prokaryotes contains amino sugars and muramic acid. In *eukaryotes* the cell wall, when present, does not contain these substances.

5. **Flagella.** The flagella and cilia of *eukaryotes* have a definite structure consisting of 2 central and 9 peripheral fibrils. Some *prokaryotes* have flagella, but the flagella do not have the 9+2 internal structure.

6. **Cytoplasmic streaming or amoeboid movement** may occur in eukaryotic cells but does nos occur in prokaryotes.

7. **Photosynthetic apparatus.** In *prokaryotes* chlorophyll, when present, is associated with lamellae. These lamellae are, however, not enclosed by membranes and hence no distinct choloroplasts are present. In *eukaryotes*, chlorophyll, when present, is found in chloroplasts.

III. HISTORY OF CYTOLOGY

The invention of the microscope in the 17th century resulted in the beginning of a new branch of biology—*cytology*. In this section the history of cytology is being dealt with under four headings : (1) The cell and cell division, (2) cytogenetics, (3) techniques, and (4) molecular biology. The major events in cytology are given in a chronological sequence in the different sections.

(1) The cell and cell division

1658—*Jan Swammerdam* gave the first description of the cell in his account of the RBC of the frog.

1665—*Robert Hooke* discovered the cell (actually the cell wall) in sections of cork.

1745—*Charles Bonnet* discovered natural parthenogenesis, which later yielded much information on meiosis.

1772—*J. Priestly* and *J. Ingenhouz* discovered that carbon dioxide and

water are used in the presence of sunlight during photosynthesis, and oxygen is released.

1781—*F. Fontana* described the nucleolus from the skin of an eel.

1824—*P. Prevost* and *J. B. A. Dumas* described cell division for the first time by studying cleavage of the frog's egg.

1827—*Karl von Baer* discovered the mammalian ovum.

1830—*G. B. Amici* discovered fertilization in plants.

1831—*Rober Brown* described the nucleus for the first time and showed that it was present in all cells.

1835—*Felix Dujardin* described protoplasm ("sarcode") in Protozoa, and considered it to be living matter.

1838—*M. J. Schleiden* and *T. S. Schwann* formulated the cell theory,

1839—according to which all living organisms are made up of cells.

1839—*J. E. Purkinje* and *Hugo von Mohl* proposed the name protoplasm

1846 and its relationship to the cell.

1841—*Robert Remak* described amitotic cell division in the RBC of the chick embryo.

1848—*W. Hofmeister* drew figures of chromosomes of the nuclei of pollen mother cells of *Tradescantia.*

1854—*R. Virchow* gave the dictum 'omnis cellula a cellula' (all cells arise from pre-existing cells).

1870—*F. Meischer* isolated nucleoproteins from pus cells.

1873—*Anton Schneider* described chromosomes ("nuclear filaments") for the first time.

1875—*Van Beneden* observed the centriole.

1875—*O. Hertwig* showed that fertilization was the result of fusion of two cells.

1876—*L. Pasteur* discovered anaerobic release of energy from cells (fermentation) in yeasts and moulds.

1 7?—*W. Flemming* described chromatin, the deeply staining part of the nucleus.

1881—*Balbiani* discovered giant salivary gland chromosomes.

1882—*W. Flemming* gave the first accurate count of chromosomes ("nuclear filaments") and used the term "mitosis" for cell division.

1882—*W. Pfitzner* discovered chromomeres, the "granules" on the chromosomes.

1886—*C. A. MacMunn* discovered cytochrome.

1887—*E. Van Beneden* demonstrated that the number of chromosomes was halved during gamete formation, and that the chromosome number was constant for each species.

1897—*Altmann and C. Benda* observed the mitochondrion.

1898—*C. Benda* gave the name "mitochondria" to the filamentous structures found in the cytoplasm.

1898—*Camillo Golgi* gave the first clear description of the Golgi apparatus (in the nerve cells of the barn owl) by using silver impregnation methods.

1900—*J. Loeb* discovered artificial parthenogenesis by stimulating eggs to develop by chemical and mechanical methods.

1901—*T. H. Montgomery* showed that homologous chromosomes undergo pairing (synapsis) during reduction division.

1913—*L. Michaelis* and *M. Menton* showed that during enzyme action the substrate and the enzyme form an enzyme-substrate complex.

1914—*F. R. Lillie* proposed the fertilizin theory according to which fertilizin, a substance present in the jelly covering of eggs, combines with antifertilizin on the sperms resulting in clumping of sperms.

1926—*O. Warburg* discovered cytochrome oxidase, the respiratory enzyme which catalyses the oxidation of cytochromes by oxygen.

1929—*K. Lohmann* discovered ATP, the source of energy in biochemical reactions.

1931—*W. H. Lewis* discovered pinocytosis, the method by which the cell engulfs particles, often by pseudopodia formation.

1934—*R. R. Bensley* and *H. L. Hoerr* isolated mitochondria from the cell.

1935—*W. M. Stanley* isolated the tobacco mosaic virus in crystalline form.

1937—*Hans A. Krebs* discovered the citric acid cycle which was named after him.

1941—*A. Claude* isolated the mitochondria by ultra-centrifugation.

1944—*C. F. Robinow* demonstrated the nucleus in bacteria.

1945—*K. R. Porter* discovered the endoplasmic reticulum.

1945—*F. Lipmann* discovered coenzyme A, a key compound in cell metabolism.

1952—*C. De Duve* identified the lysosome.

1952—*G. E. Palade* analysed the fine structure of mitochondria and showed the presence of mitochondrial cristae.

1960—*Park* and *Pon* discovered quantasomes in the chloroplast.

1963—*Chance* and *Parsons*, *Smith* and *H. Fernandez-Moran* discovered elementary particles in the mitochondrion.

(2) Cytogenetics

1763—*J. G. Koelreuter* discovered quantitative inheritance by his observation that certain plant hybrids had characters intermediate between those of the two parents.

1866—*E. Haeckel* put forward the hypothesis that the transmission of inheritance took place through the nucleus.

1866—*Gregor Mendel* formulated the laws of heredity by his now famous work on peas.

1883—*W. Roux* proposed the role of chromosomes in heredity.

1885—*O. Hertwig* and *E. Strasburger* proposed the role of the nucleus in heredity.

1901—*Hugo de Vries* proposed the mutation theory of evolution on the basis of his observation of sudden appearance of new characters in the evening primrose *Oenothera lamarckiana*.

1902—*C. E. McClung* discovered sex chromosomes in the grasshopper.

1903—*W. S. Sutton* showed that in diploid cells chromosomes occur in homologous pairs.

1906—*W. Bateson* and *R. C. Punnett* discovered linkage of hereditary units.

1909—*F. A. Janssens* proposed the chiasmatype theory, according to which exchange of chromosomal segments of homologous chromatids takes place through chiasmata formation.

1910—*T. H. Morgan* discovered sex linkage in *Drosophila*, and in subsequent years established the gene theory.

1913—*A. H. Strutevant* built up the first chromosome map (in *Drosophila*) in which the relative positions of the genes on the chromosomes was indicated.

1914—*G. H. Shull* proposed the concept of heterosis, according to which hybrids between two races how greater vitality and vigour than their parents.

1916—*G. B. Bridges* discovered nondisjunction in the chromosomes of *Drosophila*, in which both chromosomes of a pair sometimes pass to the same cell during meiosis.

1916—*H. Winkler* discovered the concept of heteroploidy, in which deviations from the normal chromosome number take place.

1922—*C. B. Bridges* proposed the balance theory of sex, according to which sex (in *Drosophila*) is determined as a result of a balance between the autosomes and the X heterosomes.

1924—*G. D. Karpechenko* experimentally developed a new species (*Raphanobrassica*) with 18 chromosomes by crossing the radish (*Raphanus :* 9 haploid chromosomes) with the cabbage (*Brassica:* 9 haploid chromosomes).

1927—*H. J. Muller* artificially induced mutation by subjecting *Drosophila* to X-ray irradiation, and thus laid the foundation for the study of mutations,

1928—*F. Griffith* discovered genetic transduction in bacteria.

1931— *C. Stern, H. Creighton* and *B. McClintock* cytologically demonstrated crossing over.

1937—*A. F. Blakeslee* aritificially produced polyploidy by blocking cell division with the drug colchicine.

1941—*G. W. Beadle* and *E. L. Tatum* induced biochemical mutation in the bread mould *Neurospora* by X-ray irradiation.

1943—*T. .M Sonneborn* discovered cytoplasmic or extranuclear inheritance in *Paramecium.*

1944—*O. T. Avery, C. M. MacLeod* and *M. McCarty* discovered transformation in bacteria.

1949—*M. L. Barr* and *E. C. Bertram* discovered sex chromatin (Barr-body).

1956—*J. H. Tjio* and *A. Levan* give the first correct human chromosome count (46 chromosomes in the diploid condition).

1959—*C. E. Ford, P. A. Jacob* and *J. H. Tjio* discovered the chromosomal basis of certain genetic abnormalities.

(3) Techniques

1906—*M. Tswett* discovered chromatography.

1912—*A. Carrel* discovered the technique of tissue culture by which living cells can be cultivated outside the body in nutrient media.

1923—*G. Hevesy* discovered the technique of isotopic tracing in which the fate of labelled isotope molecules can be traced through a metabolic pathway.

1923—*O. Warburg* discovered a method of measuring gaseous exchange in living tissue by manometry.

1924—*A. Feutgen* discovered the test for locating DNA in the cell.

1938—*T. Svedberg* developed the technique of ultracentrifugation by which cell constituents could be separated on the basis of different densities.

1943—*A. Cluude* isolated cell components like ribosomes, mitochondria and nuclei in relatively pure form by differential ultracentrifugation.

1948—*G. H. Hogeboom,* W. *C. Schneider* and *G. E. Palade* isolated mitochondria from the cell by ultracentrifugation. and thus laid the foundation for the study of enzymatic activity in cell respiration.

(4) Molecular Biology

1940—*M. Kunitz* crystallized ribonuclease.

1949—*L. Pauling* demonstrated that protein structure is under genic control by studying haemoglobin of sickle-cell anaemia patients.

1950—*I. Caspersson* and *Brachet* showed the role of RNA in protein synthesis.

1950—*E. Chargaff* discovered that in DNA the amount of purines is equal to the amount of pyrimidines.

1952—*M. Chase* and *A. D. Hershey* showed that the gene was DNA.

1953—*J. D. Watson* and *F. C. Crick* elucidated the chemical structure of DNA, a breakthrough in molecular biology.

1954—*F. Sanger* gave the first complete structure of a protein molecule when he worked out the structure of the insulin molecule.

1955—*H. Fraenkel-Conrat* and *R. C. Williams* analysed the chemical nature of the virus (TMV) and showed that it was made up of RNA and protein.

1955—*A. Kornberg* and *S. Ochoa* biologically synthesized nucleic acid
1957—of the bacterium *Escherichia coli.*

1956—*V. M. Ingram* traced mutation in haemoglobin of sickle-cell anaemia to a change in a particular amino acid.

1957—*Seymour Benzer* gave the concept of the cistron (gene), the unit of function.

1958—*F. H. C. Crick* proposed the central dogma of molecular biology that DNA determines the sequence of amino acids in a polypeptide.

1960—*J. Hurwitz, A. Stevens* and *S. Weiss* showed the role of the enzyme RNA polymeraee in the synthesis of RNA from a DNA template.

1961—*F. H. C. Crick* and others produced direct evidence that the genetic code is a triplet one.

1961—*F. Jacob* and *Monod* discovered regulatory genes.

1963—*H. M. Temin* discovered RNA-directed DNA synthesis in certain tumour viruses.

1964—*R. M. Holley* described the nucleotide sequence of alanine tRNA molecule of yeast.

1970—*H. Khorana* synthesized an artificial gene from DNA nucleotides.

2

PROTOPLASM

Protoplasm has been defined as the 'material basis of life'. The term 'protoplasm' was coined in 1839 by the Bohemian physiologist Johannes Purkinje. Protoplasm refers to the substance of which the cell is made and includes all parts of the cell. It is considered to be a living substance since it metabolizes and self-perpetuates. The term *deutoplasm* is used to describe the substances formed by the protoplasm. Protoplasm is divided into *nucleoplasm* or protoplasm of the nucleus and *cytoplasm* or extra-nuclear protoplasm.

Protoplasm is made up mainly of oxygen, carbon, hydrogen and nitrogen, which make up 95% by weight of the body. It also has a group of *minerals*. Protoplasm is made up of both inorganic and organic substances. Water, the main inorganic substance, varies from 5% to 90% in different tissues, with an average of 65% to 75%. Table 2.1 shows the proportion of various elements in protoplasm.

Table 2.1. The proportion of various elements in protoplasm.

Element	*Weight %*
Oxygen	62.00
Carbon	20.00
Hydrogen	10.00
Nitrogen	3.00
Calcium	2.50
Phosphorus	1.14
Chlorine	0.16
Sulphur	0.14
Potassium	0.11
Sodium	0.10
Magnesium	0.07
Iodine	0.014
Iron	0.010
Trace elements	0.756

Colloidal nature of protoplasm

Wilson (1925) proposed the *colloidal theory* for the organisation of the cell substance. Proteins of the protoplasm are present in a colloidal suspension. Colloidal particles are similar to ions or molecules in solution in that they do not settle down. They are however, much larger than ionic or molecular particles, being 0.1 to 0.0001 μ in diameter, and are visible under the ordinary microscope.

The protein molecules are solid particles in the *disperse phase*, and are suspended in a watery dispersion medium or *continuous phase*. Protoplasm has several properties of colloids.

(1) Colloidal particles are in a constant state of motion. The movement, which can be seen under the microscope, is called *Brownian movement*, after its discoverer Robert Brown. The intensity of the Brownian movement depends upon temperature, size of the particles and viscosity.

(2) Colloidal particles have the property of scattering light. When a beem of light is passed through a colloidal solution it becomes visible (*Tyndall effect*). A colloidal solution of proteins in water shows a typical Tyndall cone.

(3) *Sol and Gel states.* Colloidal systems have the property of undergoing changes in consistency or rigidity. A colloidal suspension can be watery at one time and jelly-like at another. For example gelatin is watery when warm but becomes jelly-like when cooled. The watery condition is called the *sol* state and the semi-solid condition the *gel* state. The two states are reversible. In the sol state the solid particles of the disperse phase are separate or discontinuous, while the fluid of the dispersion medium is continuous. In the *gel* state the solid particles are continuous, while the watery phase is discontinuous (Fig. 2.1). In both cases there is no change in the size, number or nature of the particles. Both sol and gel states are found in protoplasm.

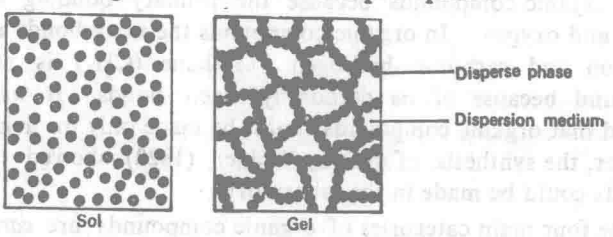

Fig. 2.1 Colloidal nature of protoplasm.

The plasma membrane and the other cell membranes are colloidal gels. The major part of the cytoplasm is largely in the gel state. If the cell membrane is punctured, the wound heals under certain condi-