THE CELL
AND
MOLECULAR
BIOLOGY:
FUNDAMENTAL
AND
APPLICATIONS
THE CELL AND MOLECULAR BIOLOGY
Fundamentals and Applications

S. S. Purohit
Ex-Head, P. G. Department of Botany
Dungar College, Bikaner

AGROBIOS (INDIA)
Molecular biology is the study of biology at a molecular level. The field overlaps with other areas of biology and chemistry, particularly genetics, biochemistry and biotechnology. Molecular biology chiefly concerns itself with understanding the interactions between the various systems of a cell, including the interactions between DNA, RNA and protein biosynthesis and learning how these interactions are regulated.

William Astbury described molecular biology as: "... not so much a technique as an approach, an approach from the viewpoint of the so-called basic sciences with the leading idea of searching below the large-scale manifestations of classical biology for the corresponding molecular plan. It is concerned particularly with the forms of biological molecules and ..... is predominantly three-dimensional and structural - which does not mean, however, that it is merely a refinement of morphology - it must at the same time inquire into genesis and function." [W.T. Astbury, Nature 190, 1124 (1961)].

Molecular biology is the study of molecular underpinnings of the process of replication, transcription and translation of the genetic material. The central dogma of molecular biology where genetic material is transcribed into RNA and then translated into protein, despite being an oversimplified picture of molecular biology, still provides a good starting point for understanding the field. This picture, however, is undergoing revision in light of emerging novel roles for RNA.

Much of the work in molecular biology is quantitative, and recently much work has been done at the interface of molecular biology and computer science in bioinformatics and computational biology. As of the early 2000s, the study of gene structure and function, molecular genetics, has been amongst the most prominent sub-field of molecular biology.

Increasingly many other fields of biology focus on molecules, either directly studying their interactions in their own right such as in cell biology and developmental biology, or indirectly, where the techniques of molecular biology are used to infer historical attributes of populations or species, as in fields in evolutionary biology such as population genetics and phylogenetics. There is also a long tradition of studying biomolecules "from the ground up" in biophysics.

Researchers in molecular biology use specific techniques native to molecular biology, but increasingly combine these with techniques and ideas from genetics and biochemistry. There is not a defined line between these disciplines. Today the terms molecular biology and biochemistry are nearly interchangeable.

Biochemistry is the study of the chemical substances and vital processes occurring in living organisms. Biochemists focus heavily on the role, function, and structure of biomolecules. The study of the chemistry behind biological processes and the synthesis of biologically active molecules are examples of biochemistry.
Genetics is the study of the effect of genetic differences on organisms. Often this can be inferred by the absence of a normal component (e.g. one gene). The study of mutants—organisms which lack one or more functional components with respect to the so-called "wild type" or normal phenotype. Genetic interactions such as epistasis can often confound simple interpretations of such knock-out studies.

The book describes key cell biology concepts needed to study molecular biology, and presents the key concepts of molecular biology necessary for various fields of biosciences. The book explores the basic concepts of Molecular Biology of the Cell with current understanding of cell biology and intriguing implications and possibilities of that which remains unknown. The book includes the chapter like, Cell, Cell Theory, Molecular Biology and Biochemistry: Historical Development, Origin of Life, Cell Types and Diversity, How Cell is Studied, Microscopy, Chemistry of Cell, Organic Components (Proteins, Carbohydrates, Lipids, Nucleic Acids: DNA, RNA, and Enzymes), Cell Membrane Structure and Functions, Cell Surface and Cellular Interactions, The Cytoskeleton, Mitochondria and Cell Energetics, Plastids and Chloroplasts, Endoplasmic Reticulum, Golgi Complex, Lysosomes, Ribosomes, Centrioles, Interphase Nucleus, Prokaryotic Chromosome, Plasmids, Cosmid, IS Elements, Transspones and Retroelements, Eukaryotic Chromosome, Cell Cycle: Molecular Basis, Mitosis, Meiosis, Reproduction: Germ Cells, Fertilization, Parthenogenesis and Apomixis, Basic Concepts of Genetics, Metabolism (Carbohydrates, Protein, Lipids, Nucleic Acid, Inborn Errors), Cytoplasmic Inheritance, Linkage and Crossing over in Diploid Organisms, Sexuality and Recombination In Bacteria and Viruses, Mutation (General Concepts, The Molecular Basis, Variation In Chromosome Number and Structure, in Microorganisms, Inheritance), The Gene, Gene Expression, Gene Cloning And Recombinant DNA Technology, DNA Fingerprinting, Expression of Induced Genes, Gene Silencing and Antisense Technology, Genetic Engineering Techniques, Gene Therapy, Immunology and Serology, Hybridoma Technology and Monoclonal Antibodies, Cellular Aging and Senescence and Neoplasia with clear and concise concept headings introduce each section.

The book is prepared by extracting fundamental concepts and meaning from this enormous and ever-growing field, including the story of cell biology, and create a coherent framework through which non-expert readers may approach the subject. Written in clear and concise language, and illustrated. The book is enjoyable to read, and provides a sense of the excitement of modern biology.

We express our gratitude to the researchers who have done tremendous job to develop the concepts of cell and molecular biology with new applications. Healthy criticism for further improvement of the book are solicited.

S. S. Purohit
3. CELL TYPES AND DIVERSITY .......................................................... 39

3.1 Diversity of Cells ............................................................................. 39
3.2 Viruses ............................................................................................ 39
3.3 Prokaryotes ..................................................................................... 41
   3.3.1 Bacteria .................................................................................. 44
   3.3.2 Blue Green Algae ................................................................. 45
3.4 Eukaryotes ....................................................................................... 46
3.5 Evolutionary Distance in Living Organisms ...................................... 53
   3.5.1 Bacterial Progenitor ............................................................... 53
   3.5.2 Example: Aquifex Bacteria ................................................... 53
   3.5.3 Example: Hydrogenobacter Bacteria ...................................... 53
   3.5.4 Microorganisms from Cell Organelle ...................................... 53
   3.5.5 Mitochondria are the Descended form of Bacteria? ............... 54
   3.5.6 Evolution of Mitochondria in Protozoa ................................. 54
   3.5.7 The Endosymbiotic Hypothesis ............................................. 54
3.6 Compartments in Eukaryotic Cells .................................................. 54

4. HOW CELL IS STUDIED .................................................................... 56

4.1 Cytochemistry ................................................................................ 56
   4.1.1 Detection of Proteins by Million’s Reaction ......................... 56
   4.1.2 Detection of Polysaccharides by Periodic Acid-schiff (PAS) Reaction ........................................................................... 57
   4.1.3 Detection of Lipids by Sudan Black B Staining ..................... 57
   4.1.4 Detection of DNA and RNA by Acridine Orange Staining ...... 58
   4.1.5 Detection of DNA and RNA by Methyl- Green- Pyronin Y Method .................................................. 58
4.2 Cell Fractionation and Centrifugation .............................................. 58
   4.2.1 Cell Fractionation Method ....................................................... 58
4.3 Cytophotometry and Cytofluorometry ............................................. 62
4.4 Autoradiography ............................................................................ 64
4.5 Immunofluorescence ..................................................................... 64
4.6 Freeze Fracture Replication and Freez Etching ............................... 65
4.7 X-Ray Diffraction .......................................................................... 69
   4.7.1 Diffraction Analysis ................................................................. 70
4.8 Cell Culture ..................................................................................... 73
   4.8.1 Biology of Cells in Culture ................................................... 74
4.9 Separatory Methods ....................................................................... 76
5. MICROSCOPY ................................................................. 95

Historical Background ........................................................................... 95
The Microscope ...................................................................................... 97
The Theory of Light Microscopy ............................................................ 97
The Wave-Particle Nature of Light; Phenomenons of Diffraction and Image
Formation .............................................................................................. 99
Optical Microscope ................................................................................ 100
The Components of the Optical Microscope ........................................... 101
General Principles of Microscopy ............................................................ 104
Brightfield Microscopy .......................................................................... 104
Polarization Light Microscopy ................................................................ 108
Optical Microscopy Techniques ............................................................. 108
Dark Field Optical Microscopy .............................................................. 109
Phase Contrast Optical Microscopy ......................................................... 109
Differential Interference Contrast Microscopy ........................................ 110
Fluorescence Microscopy ....................................................................... 110
Confocal Laser Scanning Microscopy ....................................................... 111
Deconvolution Microscopy ................................................................... 111
Sub-diffraction Microscopy Techniques .................................................. 111
Near-field Scanning Optical Microscopy NSOM ...................................... 111
Extensions of the Optical Microscope ..................................................... 112
Stereo Microscope .................................................................................. 112
The Nomarski Interference-contrast Microscopy ...................................... 113
Electron Microscopy .............................................................................. 115
Transmission Electron Microscopy (TEM) ................................................. 117
Scanning Electron Microscopy ............................................................... 119
Scanning Probe Microscope .................................................................. 120
Transmission Electron Microscope (TEM) ................................................. 122
Cryo-electron Microscope (Cryo-EM) ...................................................... 122
Ultrasonic Force Microscopy .................................................................. 123
Video Microscopy .................................................................................. 123
X-Ray Microscope ................................................................................ 124
Live-Cell Imaging Techniques .............................................................. 125
Maintaining Live Cells on the Microscope Stage ................................... 125
Live-Cell Imaging Culture Chambers ..................................................... 125
The Automatic Microscope: Shutters, Filter Wheels, Focus, Stage Control, and
Illumination Systems ............................................................................. 126
Imaging Parameters for Fluorescent Proteins ........................................ 126
Selected Literature References .............................................................. 127
6. CHEMISTRY OF CELL ......................................................... 128

   INTRODUCTION .................................................................................................................. 128
   Inorganic Components ......................................................................................................... 128
   Water ...................................................................................................................................... 128
   Minerals ................................................................................................................................. 129
   Organic Components ............................................................................................................. 130

7. ORGANIC COMPONENTS: PROTEINS ............................................. 131

   Classification of the Proteins ............................................................................................... 131
   Functional Classification of Proteins ...................................................................................... 131
   Classification on the Basis of the Shape of the Molecule .................................................... 132
   Classification Based on their Composition .......................................................................... 132
   Derived Proteins .................................................................................................................. 134
   The Amino Acids .................................................................................................................. 135
   Classification ....................................................................................................................... 135
   Ramachandran Plot ............................................................................................................... 140
   Physical Properties of Amino Acids ...................................................................................... 145
   Solubility .............................................................................................................................. 145
   Melting Point ......................................................................................................................... 145
   Taste ....................................................................................................................................... 145
   Amphoterie (or Zwitterions) ................................................................................................. 145
   Optical Activity ...................................................................................................................... 145
   Isoelectric Point .................................................................................................................... 146
   Protein Structure .................................................................................................................. 147
   Primary Structure ................................................................................................................ 147
   Secondary Structure .......................................................................................................... 149
   α-Helix Structure ................................................................................................................. 150
   Pleated Sheet Structure ....................................................................................................... 151
   Collagen Group .................................................................................................................... 152
   Tertiary Structure ................................................................................................................ 153
   Quaternary Structure .......................................................................................................... 155
   Properties of the Proteins ..................................................................................................... 157
   Taste ...................................................................................................................................... 157
   Shape ..................................................................................................................................... 157
   Molecular Weight ................................................................................................................. 157
   Amphoterie (or Zwitterions) and the Isoelectric Point ........................................................ 157
   Crystallization ....................................................................................................................... 158
   Viscosity ............................................................................................................................... 158
   Solubility and Salting Out .................................................................................................... 159
   Precipitation ......................................................................................................................... 159
   Heat Coagulation ................................................................................................................ 159
   Denaturation ......................................................................................................................... 160