



P.K.GUPTA

**CELL
AND
MOLECULAR
BIOLOGY**

Contents

1. Cell Theory and The Cell	1-8
The Cell Theory 1	
Discovery of cell and the cell theory 1	
Exceptions to the cell theory 1	
Cell Shape, Cell Size and Cell Number 3	
Cell shape 3	
Cell size 3	
Cell number 4	
	Prokaryotic Cells vs Eukaryotic Cells 4
	Eukaryotic Cells are Organized into Compartments 8
2. Techniques for Cell Study	9-27
Light Microscopy 9	
Magnification and the resolving power 10	
Fixation, sectioning and staining of tissues for light microscopy 11	
Fluorescent microscopy, phase contrast microscopy, interference-contrast microscopy and dark field microscopy 12	
Electronic imaging systems and image processors 13	
Confocal scanning light microscopy 13	
Electron Microscopy 14	
Preparation of specimens for electron microscopy (fixation, sectioning, staining, shadowing, negative staining, etc.) 14	
Transmission electron microscopy (TEM)	
Scanning electron microscopy (SEM) 16	
Freeze-fracture and freeze-etch electron microscopy 17	
Cryoelectron microscopy 17	
Cell Fractionation Methods 18	
Preparative ultracentrifugation 18	
Velocity sedimentation vs equilibrium density gradient sedimentation 19	
	Chromatography 19
	Adsorption chromatography 20
	Ion exchange chromatography 20
	Gel filtration chromatography 20
	Affinity chromatography 20
	Electrophoresis 21
	SDS polyacrylamide-gel electrophoresis (SDS-PAGE) (one dimensional) 22
	Two-dimensional PAGE 22
	X-Ray Diffraction and NMR Spectroscopy 24
	Radioisotope Tracer Technique and Autoradiography 25
	Intracellular Electrodes, and Light-Emitting Indicators 25
	Introducing Molecules into Cells for Studies of Intracellular Dynamics 26

3. Chemistry of the Cell

1. Micromolecules (Sugars, Fatty Acids, Amino Acids, Nucleotides and Lipids)

Sugars (Carbohydrates) 28

Fatty Acids 31

Amino Acids 32

Nucleotides 35

Lipids 36

Fats and waxes 36

Glycerophospholipids (phosphatides) 38

Glycosylglycerides 38

Acyl sphingosines, sphingoglycolipids and sphingophospholipids 38

Steroids and sterols 39

Ether lipids (plasmogens) 39

Prostaglandins 40

4. Chemistry of the Cell

2. Macromolecules (Nucleic Acids, Proteins and Polysaccharides)

Covalent and Weak Non-Covalent Bonds in Macromolecules 42

Ionic interactions 43

Hydrogen bonds 43

Van der Waals bonding 44

Hydrophobic bonds 45

Nucleic Acids 45

Nucleic acids as genetic material 45

Structure of nucleic acids (polynucleotides) 48

Proteins 54

Protein structure 54

Structure of proteins and weak bonds 56

Ramachandran plots and protein folding 56

Protein conformation based inheritance : the prions 56

Polysaccharides 60

Homopolysaccharides (homoglycans) 60

Heteropolysaccharides (heteroglycans) 61

Glycoproteins and glycolipids 62

5. Enzymes and Energy Transfers during Metabolism

Enzymes and Coenzymes 64

Free Energy in Cells 66

Energy Transfers and ATP (Solar Energy and Cellular Energy) 68

NADH as Intermediate Between Glycolysis / Citric Acid Cycle and Oxidative Phosphorylation to Produce ATP 69

6. Cell Wall and Extracellular Matrix (ECM)

Extracellular Matrix in Bacteria and Plant Cells : The Cell Wall 72

Cell wall in bacteria 73

Cell wall in higher plants 73

Extracellular Matrix (ECM) in Animal Cells 78

Formation of extracellular matrix 78

Glycosaminoglycans (GAGs) and proteoglycans 79

Fibrous structural proteins 82

Fibrous adhesive proteins 84

Basal lamina and ECM 86

7. Cytoskeleton : Microtubules, Actin Filaments and Intermediate Filaments

88-100

Microtubules 88

- Structure of microtubules 88
- Chemical composition and assembly 89
- Microtubules in cilia and flagella 90
- Microtubules in cell division 91
- Motor proteins in membrane traffic 93

Actin Filaments or Microfilaments 95

- Actin filaments in muscle cells (including muscle contraction) 95
- Actin filaments in non-muscle cells 96
- Actin cytoskeleton is linked with surface receptors through Rho GTPases 97

Intermediate Filaments 98

- Structure and chemistry of intermediate filaments 98
- Four types of intermediate filaments 98
- Connections between IF network and actin filaments or microtubules 98
- Intermediate filaments during mitosis 99
- Functions of intermediate filaments and their relation to genetic diseases 99

8. Cell Membrane (Including Plasma Membrane)

101-119

Isolation and Chemical Composition 101

- Lipids as phospholipids, glycolipids and cholesterol 102

Membrane Proteins 105

- Segments spanning the membrane and those outside the membrane 106
- Glycosylation and disulphide bonds on the non-cytosolic side of transmembrane proteins 107
- Three representative membrane proteins spectrin, glycophorin and band 3) from RBCs 107
- Bacteriorhodopsin (from bacteria, *Halobacterium halobium*), a seven α helices transmembrane segments (TMS) protein, is a proton pump 110
- Porins (transmembrane proteins) cross the membrane as β barrel 110

Protein Complexes in Membranes 110

Specific Proteins for Specific Membrane Domains 111

- Cell coat or glycocalyx (rich in sugar residues) 111
- CAMs (cadherins and selectins) and integrins as membrane proteins 111

Membrane Skeleton (Plasma Membrane Cytoskeleton Interaction) 112

Organization of Lipids and Proteins

(Models for Structure of Membrane) 112

- Artificial membrane systems 112
- Historical models for structure of plasma membrane 113
- Universally accepted fluid mosaic model for plasma membrane 114
- Modification of fluid mosaic model 118
- Fluidity of bilayer depends on its composition (hydrocarbon chains and cholesterol) 119

9. Cell Organelles

120-127

I. Mitochondria

Morphology 120

Ultrastructure 121

Isolation of Mitochondria and Mitochondrial Membranes 122

Chemical Composition 123

Mitochondrial Genome 123

Transport of Proteins into Mitochondria 124

- Transport of proteins to mitochondrial matrix 124
- Transport of proteins into the inner membrane and intermembrane space of mitochondria 125

Function 126

Inheritance of Mitochondria 126

Evolutionary Origin of Mitochondria 126

- Monophyletic origin of mitochondrion from a free living proteobacterium 126
- Simultaneous origin of eukaryotic cells and mitochondria ('serial endosymbiosis' vs 'hydrogen hypothesis') 127

10. Cell Organelles

128-1

2. Plastids

- Chloroplasts 128
 - Shape, size and number 129
 - Ultrastructure 129
 - Isolation and chemical composition 131
 - Transport of proteins into chloroplasts 132
 - Function 133
 - Chloroplast genome and autonomy of chloroplasts. 133

- Leucoplasts and Starch Granules 134
 - Structure and function 134
 - Origin 134

- Chromoplasts 134
 - Structure and function 134
 - Origin from chloroplasts 134
 - Origin from leucoplasts 135

Evolutionary Origin of Plastids from Cyanobacteria and "Endosymbiotic Gene Transfer" 135

3.

11. Cell Organelles

137-1

3. ER, Ribosomes, Endosomes, Golgi, Lysosomes, Peroxisomes, Hydrogenosomes and Centrosome

- Cytoplasmic Matrix (Cytosol) 137

- Endoplasmic Reticulum (ER) 138
 - Ultrastructure 138
 - Isolation and chemical composition 139
 - Import of proteins into ER (ER signal peptide, signal recognition particle (SRP) and SRP receptor) 139
 - Functions 140
 - Origin 141

- Ribosomes 142
 - Ultrastructure 142
 - Chemical composition and organization 142
 - High resolution structure (atomic level) of ribosome using cryoelectron microscopy (cryo-EM) and X-ray crystallography 144
 - Function (ribosome as a ribozyme-peptidyl transferase) 145
 - Biogenesis 146

- Golgi Complex 146
 - Ultrastructure 146
 - Isolation and chemical composition 147
 - Function 147
 - Origin 147

- Endosome 147
 - Structure and chemical composition 147
 - Types of endosomes 148
 - Function 148

- Lysosomes 148
 - Structure and occurrence 148
 - Isolation and chemical composition 148
 - Types of lysosomes 149
 - Functions 149
 - Origin 151

4.

- Peroxisomes 151
 - Morphology 151
 - Import of proteins into peroxisomes using a short signal sequence 151
 - Function 151
 - Origin 153

- Hydrogenosome 153
- Centrosome 153
 - Structure 153
 - Function 154
 - Centrosome and cancer 154
 - Origin 154

5.

12. The Cell Nucleus

155-17

- Nuclear Envelope 156
 - Disassembly and reassembly of nuclear envelope 156
 - Nuclear pore complex (NPC) 157
 - Nucleocytoplasmic transport 157
 - Evolutionary origin of nucleus 160

- Nucleolus 161
 - Nucleologenesis 161
 - Nucleolus and the ribosome biogenesis 162
 - Small nucleolar RNAs (snoRNAs) and rRNA processing 162

Chromosomes 163

- Number, size and shape of chromosomes 163
- Morphology of chromosomes 164
- Karyotypes 164
- Euchromatin and heterochromatin 166
- Constitutive and facultative heterochromatin 167
- Single-stranded and multi-stranded hypotheses for chromosomes 167
- Chemical composition of chromosomes 167
- Ultrastructure of chromosomes 168

- Structure of chromosomes at molecular level 169
- Function of chromosomes 172
- Special types of chromosomes 172

Prokaryotic Nucleoids (Bacterial and Plasmid Genomes) 175

- Bacterial genome 175
- Plasmid genome 176
- Centromere and telomere like structures in bacteria and plasmids 176

13. Energy Conversions : Photosynthesis and Respiration 177-222**Photosynthesis 178**

- Photosynthetic apparatus 179
- Light harvesting complexes or antenna complexes (LH1 and LH2 in bacteria; LHCI and LHCII in plants) 181
- Reaction centres for photosynthesis and their evolutionary origin 183
- Mechanism of photosynthesis 187
- Light induced electron transport and generation of NADPH and ATP 187
- Cyclic photophosphorylation and production of ATP without the concurrent production of NADPH 193
- Carbon fixation during dark reaction 194

Photorespiration and Associated

- Harmful and Useful Effects on Yield 202
- Mechanism of photorespiration and harmful attributes 202
- Useful attributes of photorespiration 203

Respiration 204

- Aerobic respiration 205
- Glycolysis (EMP pathway) 207
- Fatty acid oxidation cycle 207
- Oxidative decarboxylation of pyruvate 209
- The citric acid cycle (TCA or Krebs cycle) 209
- Respiratory chain for oxidative phosphorylation 212
- Pentose phosphate (Warburg Dickens) pathway 220
- Anaerobic respiration and fermentation 220
- O₂ and CO₂ compensation points 222

14. Membrane Function**1. Cell Adhesion and Cell Junctions****Cell Sorting and Differential Cell Affinity 223**

- Cell Adhesion Molecules (CAMs) 225
 - Cadherins (Ca⁺⁺ dependent cell-cell adhesion) 225
 - Immunoglobulin superfamily CAMs (Ca⁺⁺ independent cell-cell adhesion) 227
 - Selectins 230

- Integrins (cell receptors for ECM molecules): focal adhesions and hemidesmosomes 231
- Glycosyltransferases (cell receptors for ECM molecules) 234

Cell Junctions 234

- Cell junctions in animals 234
- Cell junctions in plants (plasmodesmata) 238

15. Membrane Function**2. Membrane Transport and Membrane Excitability (Neurotransmission)****Membrane Transport Common to all Eukaryotes 243**

- Passive transport : passive diffusion 243
- Passive transport : transport of ions across an ion gradient 244
- Passive transport: facilitated diffusion 248
- Active transport 251

Membrane Transport in Bacteria (*E. coli*) 256

- Channels in outer membrane 256
- Plasma membrane transporters 256

Membrane Excitability in Animals

- (Neurotransmission and Ion Channels) 257
- Neurons and neuroglia (or glial cells) 258
- Ion gradients for transmission of nerve impulse or action potential 259
- Myelination and propagation of nerve impulse 261
- Cell - cell communication at synapse 262

223-240

241-265

16. Membrane Function**3. Vesicular Transport and Membrane Fusion
(Secretory and Endocytic Pathways)**Protein Sorting and Vesicular Traffic
from ER to Golgi 267Retrograde Transport (Golgi to ER and
ER to Golgi) 269Transport from Cis-Golgi to Trans-Golgi :
Flow Pattern in Golgi Stack, as a
Distillation Tower (Anterograde and
Retrograde Transport) 270Transport from the Trans-Golgi Network
(TGN) to Lysosomes 270Transport from Trans-Golgi Network to
Cell Surface (Exocytosis):
Secretory Vesicles for Transport of
Secretory Proteins 271Synaptic Vesicles for Transport of
Neurotransmitters 273

Role of 'Annexins' in Exocytosis 273

Transport from Plasma Membrane via
Endosomes: Endocytosis 273Caveolae and their role in potocytosis,
transcytosis and signal transduction 274Delivery of Molecules to Lysosomes
for Digestion 275

Polarized Cells and Vesicular Transport

Molecular Mechanism of Vesicular
Transport 276

Coat proteins and coated vesicles 277

SNARE hypothesis and fusion machine

(v-SNAREs, t-SNAREs and Rab proteins) 281

Regulation of membrane traffic by
phosphoinositides 283**17. Cell Division (Mitosis and Meiosis)**

287-29

Mitosis 287

Cell cycle (interphase and mitosis) 287

Cytokinesis 289

Meiosis 290

Significance of meiosis 290

Reproductive cycles 290

Stages of meiosis 291

Post-reductional or inverted meiosis and chiasma
terminalization 295Synaptonemal complex (SC) and its significance
in meiosis 295Recombination nodules and their role in meiotic
recombination 297

Comparison of Meiosis and Mitosis 297

18. The Cell Division Cycle : Molecular Basis

299-32

Genetic vs Biochemical Approaches
for the Study of Cell Division Cycle 299

Mitotic Cell Division in Yeast 300

Variation in Mitotic Cell Cycle 301

The Central Cell Cycle Control System 302

Cell Cycle Checkpoints 303

Feedback Controls and Inhibitory Signals 304

Cyclin Dependent Kinases (Cdks) and
Cyclins (Cell Cycle's Engine) 304

CDKs and cyclins in fission yeast 306

CDKs and cyclins in budding yeast 308

Cdks and cyclins in mammalian cells 309

Association of two alternative forms of Cdk
(M and S forms) with M cyclins
and G1 cyclins 310Reversible phosphorylation of CDKs,
and CDK activation kinases (Caks) 310

Cdk inhibitors (CKIs) and Suc1 (Cks1) 311

Interaction of cdc6p, cdc18p and rum1
with CDKs 312Dependence of mitosis on DNA replication
and DNA repair 313Dependence of centrosome duplication
on DNA synthesis 315

Dependence of mitosis on spindle function 315

Dependence of mitosis on growth 317

Cell Division Controls in Multicellular
Animals 317

The Cell Cycle and Cancer	318
Cyclins (cyclin D1, cyclin E, cyclin A) and cdk's in cancer	318
Cell cycle inhibitors and cancer	319
Tumour suppressors (p53 and Rb)	319
Aneuploidy and Cancer	319
Meiotic Cell Division	319
Recombination precedes synapsis in yeast	319
Drosophila genes for meiosis	319
Yeast genes for meiosis	320
Role of MPF during meiosis	320
Biochemistry of chromosome pairing in meiosis	321

Dynamics of Chromosome Movements During Cell Division	322
Events involving chromosome movement	322
Kinetochores and spindle in chromosome movement	322
Basic questions about kinetochore function	323
<i>In vitro</i> association and relative movement of microtubules and kinetochores	324
Role of Degradation of Proteins (Proteolysis) in Cell Cycle	325
Factors determining which proteins to be degraded and when	326

9. Germ Cells, Fertilization, Parthenogenesis and Apomixis

328-354

Reproduction in Animal Systems	328
Germ cell determination by chromosome diminution	328
Germ cell determination due to germinal poleplasm	329
Gametogenesis	331
Structure of gametes	335
Recognition of egg and sperm	336
Egg-sperm membrane fusion and prevention of polyspermy	338
Egg metabolism during fertilization	339
Rearrangement of egg cytoplasm after fertilization	339
Fusion of the genetic material	340
Non-equivalence of mammalian pronuclei	341
Parthenogenesis	341

Reproduction in Plant Systems	343
Anther and pollen	344
Ovule and embryo sac	346
Cellularization of embryo sac	348
The egg cell	349
Receipt of the pollen tube, release of sperms and double fertilization	349
Genetic and molecular analysis of ovule and embryo sac development	350
Apomixis	350
Molecular mechanisms for sexual and apomictic developmental pathways	353

20. Basic Concepts in Genetics

355-368

Mendel's Work	355
Principle of segregation (Law of purity of gametes or Mendel's first law)	356
Principle of independent assortment (Mendel's second law)	357
Mendel's Results, Chromosome Theory and Linkage (Present Status)	358
Molecular Basis of Mendel's Wrinkled Seed Character	359
Mendelian Genetics in Humans	359
Interaction of Genes	360
Modifiers, Suppressors and Pleiotropic Genes	361

Meiotic Drive, Segregation Distortion and Selfish Genes	361
Penetrance and Expressivity	362
Multiple Factors or Polygenes	362
Multiple Alleles and Isoalleles	364
Sex Linkage	365
Non-disjunction of sex chromosomes in <i>Drosophila</i>	366
The Chromosome Theory of Inheritance	367
Forward Genetics vs Reverse Genetics	367

21. Maternal Effects and Cytoplasmic Inheritance 369

- Maternal Effects 370
 - Pigment in flour moth (*Ephesia kuehniella*) 370
 - Coiling of shell in snail (*Limnaea peregra*) 371
- Cytoplasmic Inheritance Involving Dispensable Hereditary Units 371
 - (nuclear genes may or may not be involved)
 - Kappa in *Paramecium* 371
 - CO₂ sensitivity in *Drosophila* (sigma factor) 373

- Organelle Genetics 373
 - Plastid inheritance: variegation in plants 373
 - Male sterility in plants 375
 - Chloroplast genetics 378
 - Mitochondrial genetics 383
 - Paternal inheritance of cpDNA and mtDNA

22. Linkage and Crossing Over in Diploid Organisms (Higher Eukaryotes) 391

- Coupling and Repulsion Hypothesis 391
- A Testcross in Maize 393
- Crossing Over and Meiosis 393
 - Crossing over and chiasma formation 393
 - Mechanism of genetic recombination 394
- Crossing Over and Linkage Maps 396
 - Recombination frequencies from a test-cross (a three-point testcross) 396
 - Recombination frequencies from F₂ data 398
 - Prediction of F₂ ratios for linked genes 398
 - Interference and coincidence 399
 - Linkage maps 400
 - Mapping function and Poisson distribution 400

- Linkage groups 404
- Complete linkage in *Drosophila* males 404
- Chi square (χ^2) test for segregation ratios and detection of linkage 404

- Cytological Basis of Crossing Over 406
 - Stern's experiment in *Drosophila* 407
 - Creighton and McClintock's experiment in corn 409
 - Meselson and Weigle's experiment using lambda (λ) phage 410

- Crossing Over at Four Strand Stage 410

23. Tetrad Analysis, Mitotic Recombination and Gene Conversion in Haploid Organisms (Fungi and Single Celled Algae) 412-419

- Tetrad Analysis 412
 - Analysis of ordered tetrads 414
 - Analysis of unordered tetrads 418
 - Tetrad analysis in higher plants (a novel technology) 419

- Mitotic Recombination and Chromosome Mapping 419
 - Somatic crossing over in *Drosophila* 419
 - Mitotic recombination and parasexual cycle in *Aspergillus* 421

- Gene Conversion 423

24. Sexuality and Recombination in Bacteria and Viruses 425-431

- Three Methods for Transfer of Genetic Material 425

- Sexual Conjugation In Bacteria 426
 - Culture media and mutant strains 426
 - Discovery of gene transfer 426
 - Discovery of linkage in bacteria 427
 - Donor (F⁺ or Hfr) and recipient (F⁻) strains (discovery of fertility or sex factor F) 428
 - Conversion of F⁺ into Hfr strain 430

- Fertility factor F⁺ and sexduction 430
- Mechanism of chromosome transfer 430
- Physical structures involved in chromosome transfer 430

- Retrotransfer and Conjugation 431

- Linkage Maps in Bacteria 431
 - Conjugation mapping through interrupted mating 431

Linkage information from transformation 432
 Recombination after gene transfer 432
 High resolution mapping 432
 Linear order of genes 433

Sporulation and Cell Division in Bacteria 433

Asymmetric cell division during sporulation
 in *Bacillus subtilis* 433
 Vegetative cell division in *E. coli*
 and *B. subtilis* 434

Replication and Recombination in Viruses 434

Replication of bacteriophages 435
 Lysogenic bacteria 435
 Transduction 436
 Recombination in viruses 437
 Circular genetic maps in viruses 437

25. Molecular Mechanism of Genetic Recombination 439-445

Hybrid DNA Models Involving
 Single Strand Breaks
 (e.g. Holliday Model) 439

Hybrid DNA Models Involving Double
 Strand Breaks 441

Mismatch Repair and Post-Meiotic
 Segregation (pms) in Heteroduplex Region 442

Enzymes and Proteins Involved in
 Recombination 444

26. Recombination and Resolution of Gene Structure 446-455 (A Modified Concept of Allelomorphism)

Gene vs Allele : A Modified Concept of
 Allelomorphism 446

Classical concept 446

Position effect 447

Pseudoalleles and complex loci 449

Position pseudoallelism or *cis-trans* effect 450

Fine Structure of Gene

(using recombination among mutants) 451

Fine structure of *lozenge* locus in *Drosophila* 451

Fine structure of *rII* locus in T4 phage 451

Cistron, recon and muton 455

27. Plasmids, IS Elements, Transposons and Retroelements 456-471

Plasmids 456

Classification of plasmids 457

Replication, transfer and recombination
 in plasmids 458

Insertion Sequences (IS) or IS Elements 459

Transposons and Controlling Elements

(Mediated *via* DNA) 460

Transposons in prokaryotes 460

Transposons in eukaryotes 462

Retroelements (Transposable Elements

Involving RNA Phase) 467

Retrotransposons in plants 469

Mechanism of Transposition 470

Uses of Transposons 470

Transposons as genetic markers 470

Transposons as mutagens and transposon tagging
 for isolation of genes 471

Transposons as transformation vectors 471

28. Structural Changes in Chromosomes 472-481

Deficiencies 473

Duplications 474

Translocations 475

Cytology of a translocation heterozygote 475

Breeding behaviour of a translocation
 heterozygote 476

Interchange heterozygosity in *Oenothera* 476

Inversions 477

Cytology of inversions 477

Genetic consequences of inversion 479

Overlapping inversions 479

Inversions in *Drosophila* populations 479

29. Numerical Changes in Chromosomes

Aneuploidy 482
 Monosomy 482
 Nullisomy 483
 Trisomy 483
 Tetrasomy 485

Euploidy 485
 Monoploidy and haploidy 485
 Polyploidy 487

482

30. Mutations

1. Biochemical Level (Biochemical and Microbial Genetics)

Inborn Errors of Metabolism in Man 494
 Eye Transplantation in *Drosophila* 495
 Biochemical Mutations in *Neurospora* 496
 Total isolation method 496
 Replica plating technique 497
 Filtration enrichment technique 497
 Penicillin enrichment method 498
 Mutations in *E. coli* for Resistance against
 Phages or Antibiotics 498
 Cell Counting in Suspension 498

Calculation of Mutation Rates and
 Frequencies 498

Biochemical Mutations and Biosynthetic
 Pathways 499

Arginine synthesis in *Neurospora* 499
 Synthesis of tryptophan in bacteria and
Neurospora 499

Linear Order of Genes in Genome and the
 of Enzymes in a Biosynthetic Pathway
 Tryptophan metabolism in *Salmonella* 500
 Histidine metabolism in *Salmonella* 501

494-5

3.

31. Mutations

2. Molecular Level (Mechanism)

Mutations and Nucleotide Sequences in
 Nucleic Acid 502
 Effect of chemical mutagens on nucleotide
 sequence 503
 Effect of dyes on nucleotide sequence 508
 Effect of physical conditions on nucleotide
 sequence 508
 Effect of radiations on nucleotide sequence 508
 Spontaneous mutations and nucleotide sequence 511

Mutations and Amino Acid Sequences in
 Proteins 512

Sanger's technique for amino acid sequence
 determination 512

Altered amino acid sequences in mutants 512

Suppressor Mutations, tRNAs and
 Ribosomes 514

502-5

34

32. Chemistry of the Gene : Synthesis, Modification and Repair of DNA

DNA Replication : General Features 516
 Semi-conservative DNA replication in *E. coli* 516
 Semi-conservative replication of chromosomes
 in eukaryotes 518
 Semi-discontinuous DNA replication 519
 Unidirectional and bidirectional DNA replication 520
 RNA primers in DNA replication 521
 Regulation of DNA replication by anti-sense
 RNA primers 522
 Prokaryotic DNA polymerases 522
 Eukaryotic DNA polymerases 526
 Replicons for DNA replication 526

DNA Replication in Prokaryotes 527

Experimental approaches for the study
 of DNA replication 527

Initiation of DNA replication 527

Elongation of DNA chain: replication
 elongation proteins 530

Replication fork movement 531

Termination of DNA replication 533

DNA Replication in Eukaryotes 534

DNA replication and cell cycle 534

Replication origins and initiation of DNA
 replication (cis and trans-acting elements :
 replicators and initiators) 534

516-535

Cdk2 and cyclins in DNA replication 537
 Cdc7p-Dbp4p for MCM phosphorylation 537
 Cln3-Cdc28 kinase for transcriptional control of S phase genes 538
 Comparison of initiation of DNA replication with transcription initiation 538
 Different steps involved in eukaryotic DNA replication 538
 Synthesis of telomeric DNA by telomerase 541
 Regulation of telomere length 541
 Models of DNA replication 542

RNA Directed DNA Synthesis (Reverse Transcription) 543

DNA Modification and DNA Restriction 544

DNA Repair 544
 Direct repair 545
 Excision repair systems in *E. coli* 545
 Mismatch repair in eukaryotes (yeast and humans) 547
 Relationship between NER and transcription 547
 Recombinational repair (dimer tolerance) involves DNA replication in *E. coli* 547
 An SOS repair system in *E. coli* 548
 DNA repair and genetic diseases in humans 548

33. Organisation of Genetic Material 551-561

1. Packaging of DNA as Nucleosomes in Eukaryotes

Techniques Leading to Nucleosome Discovery 552

X-ray diffraction and electron microscopy 552
 Chromatin reconstitution experiments 552
 Nuclease digestion and regular distribution of histones 552
 Distribution of histones within a repeat unit through cross linking 553

Subunit of Chromatin: The Nucleosome 554

Basic structure of a nucleosome 554
 Structural periodicity and super-coiling of DNA in a nucleosome 554
 Core particle 555

Spatial Arrangement of Histones 557

Relation Between Different Nucleosomes 558

Solenoid Model 558

Loops, Domains and Scaffolds in Chromatin 559

Chromatin Replication and Nucleosome Assembly 559

Phasing and Modification of Nucleosomes in Active Genes 560

Nucleosome phasing 560
 Ubiquitination, acetylation, methylation and phosphorylation of histones in the nucleosomes 560

34. Organization of Genetic Material 562-570

2. Repetitive and Unique DNA Sequences

Chromosomal DNA Content and C-Value Paradox 562

Repetitive DNA 563

Technique for detecting repetitive DNA 563

Chemical complexity vs sequence (kinetic) complexity 565

Repetitive DNA in the form of satellite DNA 567
in-situ hybridization of sat DNA 568
 Squash dot hybridization 569

Selfish DNA 569

35. Organization of Genetic Material 571-581

3. Split Genes, Overlapping Genes, Pseudogenes and Cryptic Genes

Split Genes or Interrupted Genes

Discovery and nature of split genes 571
 R-loop mapping and restriction mapping of interrupted genes 573
 Structure of chicken ovalbumin split gene 575
 Split genes in fungal mitochondria 575
 Split genes in chloroplasts 576
 Intron of one gene may contain exon of another gene 576
 Exon sequences are conserved, but intron sequences vary 577
 Introns with coding sequences 577

Two hypotheses for origin of introns 'early intron' and 'late intron' 577

Overlapping Genes 578

Pseudogenes (U snRNA Series, Globin Pseudogenes and Alu Family) 579

Cryptic Genes 579

Promiscuous DNA (Movement of DNA Between Mitochondria, Chloroplasts and Nucleus) 580

36. The Genetic Code

- Properties of Genetic Code 583
 - The code is triplet 583
 - The code is degenerate 583
 - The code is non-overlapping 583
 - The code is commaless 583
 - The code is non-ambiguous 583
 - The code is universal 584
- Codon Assignments 584
 - Assignment of codons with unknown sequence 584
 - Assignment of codons with known sequence 585
- Chain Initiation and Chain Termination
 - Codons 586
- Synonym Codons and Degeneracy 587

- Mutations and Genetic Code 587
 - Frameshift mutations 588
 - Base substitutions or amino acid replacement 588
- Wobble Hypothesis 588
- New Genetic Codes in Mitochondria and Ciliate Protozoa 589
- Suppression Mutations, Base Substitutions and Suppressor tRNAs 590
 - Intragenic suppression 590
 - Intergenic suppression 591
- 'Second Genetic Code' and 'Second Hand of the Genetic Code' 592
- Recoding of the Genetic Code 592
 - Alteration in the linear read-out frame 592
 - Alteration in the meaning of code words 592

37. Transfer RNA and Aminoacyl-tRNA Synthetases 595-6

- Structure of Transfer RNAs (tRNAs) 595
 - Primary structure of tRNA and modified bases 596
 - Two dimensional structure of tRNA (clover leaf model) 597
 - Three dimensional structure of tRNA 597

- Extended anticodon and translational efficiency of t-RNA 598
- Transfer RNA sequences and the origin of genetic code 599
- Aminoacyl-tRNA Synthetases (aaRs) 600
 - Interaction of tRNA and amino acyl tRNA synthetase (second genetic code) 600

38. Expression of Gene : Protein Synthesis 603-62

1. Transcription in Prokaryotes and Eukaryotes

- Transcription in Prokaryotes 604
 - Single RNA polymerase in *E. coli* 604
 - Promoter sites for initiation of transcription in prokaryotes 605
 - Initiation and elongation of RNA synthesis in prokaryotes 606
 - 'Inchworm model' for elongation of transcript 606
 - Stability of elongation complex (RNA-DNA hybrid vs RNAP-DNA interactions) 607
 - Elongation arrest vs termination of transcription 607
 - Termination and antitermination of mRNA synthesis in prokaryotes 608

- Transcription factors and initiation of RNA synthesis in eukaryotes 612
- Transcription activators and coactivators 622
- Transcription repressors and corepressors 623
- Recruitment model for transcriptional activation
- Transcription factors for elongation of RNA chain in eukaryotes 624
- Transcription slippage 624
- Termination of RNA synthesis in eukaryotes 624
- Chromatin Decompaction for Transcription in Eukaryotes 625
- Transcription in Mitochondria 626
 - Transcription of vertebrate mtDNA 626
 - Transcription of yeast and plant mtDNA 627
- Transcription in Chloroplasts 627

19. Expression of Gene : Protein Synthesis 629-641

2. RNA Processing (RNA Splicing, RNA Editing and Ribozymes)

- Polyadenylation of mRNA in Prokaryotes 629
Addition of Cap (m7G) and Tail (Poly A) for mRNA in Eukaryotes. 630
RNA Splicing 632
RNA Editing and Guide RNA 640
Ribozymes (RNA Splicing, DNA Cleavage and RNA Amplification) 641

10. Expression of Gene : Protein Synthesis 642-662

3. Synthesis and Transport of Proteins (Prokaryotes and Eukaryotes)

- Formation of Aminoacyl tRNA 642
Initiation of Polypeptide 643
Elongation of Polypeptide 649
Termination of Polypeptide 653
Translation in Chloroplasts and Mitochondria 654
Modification and Folding of Released Polypeptide 655
Protein Translocation Across Membranes 657

11. Regulation of Gene Expression 663-682

1. Operon Circuits in Bacteria and Other Prokaryotes

- Induction and Repression 663
Inducer and Co-repressor 664
The Operon Model for Transcriptional Regulation 664
Leucine Responsive Protein (Lrp) as a Global Regulator 672
Substitution of Sigma Factor and Control of Transcription 673

- DNA Sequences Controlling Transcription 674
 - Footprinting for identification of DNA sites used for protein binding 674
 - DNA sequences for CAP, RNA polymerase and lac-repressor 675
 - Identification of starting point 675
 - Pribnow box* and other sequences common to DNA regions upstream to several operons 676
- Regulation by DNA Rearrangements 676
 - Stochastic (random) gene rearrangements 676
 - Developmentally regulated gene rearrangements 676

- Post-Transcriptional Regulation 677
 - Leader sequences and attenuators 677
 - Autogenous regulation of translation 679
 - Regulation by alternative splicing 679
 - Regulation by anti-sense RNA 679
 - Repression and activation of translation 680
 - Feedback inhibition 681
- Signal Transduction and 'Two Component Regulatory Systems' 682
 - Bacterial chemotaxis 682
 - Nitrogen assimilation 682
 - Outer membrane protein expression 682

42. Regulation of Gene Expression

683-6

2. Circuits for Lytic Cycle and Lysogeny in Bacteriophages

- Regulation by a Cascade in Phages 683
- Alternative Sigma Factors in Phage SPO1 684
- Lytic Cascade in Lambda (λ) Phage 684
 - Three sets of genes in phage lambda (λ) 685
 - Anti-repressor (product of *cro* gene) for lytic cycle 687

Lysogeny through establishment and maintenance of repressor (promoters *PE* and *PM*) 687

DNA Binding of Cro and λ Repressor Proteins 690

Functional Grouping of Genes in Phages T4 and T7 690

43. Regulation of Gene Expression

692-7

3. A Variety of Mechanisms in Eukaryotes

(Including Cell Receptors and Cell Signalling)

- Regulation at Transcription Level 693
 - Activation of transcription 693
 - Repression of transcription 697
 - Specific DNA sequences controlling transcription 699
 - Transgenic plants to study regulatory sequences 702
 - Modification of DNA sequences/ transcripts and histone proteins 704
 - Alternative splicing of transcripts 705
- Regulation at Translation Level 705
 - Activation and repression of translation using mRNA binding proteins 705
 - Translational control by phosphorylation of the components of translation machinery 707
 - Masked mRNA in eggs of sea urchin and *Xenopus* 710

Regulation by Gene Rearrangement 710

- Expression of immunoglobulin genes 710
- Yeast mating type switching 712
- Trypanosome surface antigen (VSG) switching 712
- Synthesis of mRNA in pieces in VSG genes in trypanosome 714

Regulation of Gene Expression by Reversible Protein Phosphorylation 715

WD-repeat Proteins and their Regulatory Role 715

Cell Receptors, Cell Signaling and Regulation of Gene Expression 716

- Intracellular receptors 716
- Cell surface receptors 716
- Signal transduction pathways 725

44. Genetic Engineering and Biotechnology 734-754

1. Recombinant DNA, Molecular Probes, Gene Libraries,

PCR (Cloning and Amplification of DNA) and DNA Chips

Restriction Enzymes for Cloning 734

Techniques Used in Recombinant DNA Technology 735

Separation of DNA fragments using agarose or polyacrylamide gel electrophoresis 735

Separation of large DNA molecules (whole chromosomes) using PFGE 736

Southern, Northern and Western blotting 736

Dot blots and slot blots 738

Cloning Vectors for Recombinant DNA 739

Plasmids as vectors 739

Bacteriophages as vectors 741

Cosmids and phagemids as vectors 742

Plant and animal viruses as vectors 742

Transposons as vectors 742

Artificial chromosome vectors for cloning large DNA segments 742

Construction of Chimeric DNA 743

Cloning fragments with homologous cohesive ends (palindromes and staggered cleavage) 744

Directional cloning using fragments with heterologous ends 745

Adding poly dA at the 3' ends of the vector and poly dT at the 3' ends of DNA clone 745

Blunt end ligation by T4 DNA ligase 745

Cloning in Bacteria and Eukaryotes 746

Cloning in bacteria 746

Cloning in eukaryotes 746

Molecular Probes 746

Labelling of probes 747

Applications of molecular probes 748

Construction and Screening of Genomic and cDNA Libraries 748

Genomic library by shotgun experiment 748

cDNA library from mRNA 749

Colony (or plaque) hybridization for screening of libraries 749

Gene Amplification : PCR and its Applications 750

Basic polymerase chain reaction (PCR) 750

Different schemes of PCR 751

DNA Chip Technology and Microarrays 753

45. Genetic Engineering and Biotechnology 755-765

2. Restriction Maps and Molecular Genetic Maps

Restriction Mapping 756

Restriction cleavage and gel electrophoresis 756

Construction of a restriction map 757

Use of partial digests, end labeling and hybridization in restriction mapping 757

Molecular Genetic Maps 758

Restriction fragment length polymorphisms (RFLPs) 758

Random amplified polymorphic DNA (RAPD) using PCR 759

Minisatellites and microsatellites (VNTRs) 761

Sequence tagged sites (STS) and expressed sequence tags (ESTs) 761

Linkage and recombination between molecular and phenotypic markers 762

Molecular Physical Maps 762

Physical mapping using chromosome deletions 763

Physical mapping using in situ hybridization (ISH) 763

Chromosome walking for physical maps 763

Chromosome jumping (or hopping) for physical maps 764

Molecular Maps, Map-based Cloning and Reverse Genetics 764

46. Genetic Engineering and Biotechnology 766-780

3. Isolation, Sequencing and Synthesis of Genes

Isolation of Genes 766

- Early attempts for isolation of ribosomal RNA genes in *Xenopus* 766
- Isolation of genes coding for known specific proteins 767
- Isolation of genes (with known or unknown product), with tissue specific expression 768
- Isolation of genes (with known or unknown products) using DNA or RNA probes 768
- Isolation of genes coding for an unknown product 769

- Maxam and Gilbert's chemical degradation method 773
- Sanger's dideoxy nucleotide synthetic method 773
- Automatic sequencers 775

Synthesis of Genes 776

- Chemical synthesis of tRNA genes 776
- Total synthesis of a human leukocyte interferon gene 778
- Gene synthesis machines 778
- Synthesis of gene using PCR 779
- Synthesis of gene from mRNA 780

Sequencing of Gene or a DNA Segment 773

47. Genetic Engineering and Biotechnology 781-790

4. Gene Transfer Methods and Transgenic Organisms

Gene Transfer Methods 781

- Gene transfer (transfection) methods in animals 781
- Gene transfer methods in plants 783

Transgenic Organisms 786

- Transgenic animals 786
- Transgenic plants 787

48. Genetic Engineering and Biotechnology 791-799

5. Hybridoma and Monoclonal Antibodies

Hybridoma and the Production of Monoclonal Antibodies 791

- Improvements in hybridoma technology 793
- Purification of antibodies 793

Antibody Engineering and Genetic Manipulations 793

- Alternatives to hybridoma for monoclonal antibodies 794

Production of Human and Humanized Antibodies 796

- Transgenic mice for human antibodies 796
- Phage display technology and 'single pot' libraries for human antibodies 796

Uses of Monoclonal Antibodies 797

- Diagnosis, screening and therapy 797
- Vaccine production 797
- Utility as enzymes (abzymes) 797
- Purification and quantitation of other molecules 798

49. Multigene Families in Eukaryotes 800-807

How the Demand for Large Quantities of Gene Product is Met ? 800

Multigene Families 801

- How to locate multigene families ? 801

Multigene families with divergent members 801

Multigene families with identical genes 803

Concerted Evolution of Multigene Families 806

50. Specification of Cell Fate and Cell Commitment 808-816

Autonomous Specification/Determination 809

- Tunicate embryos 809
- Mollusc embryo 810
- Coenorhabditis elegans* embryo 811

Conditional Specification or Regulative Development 812

- Organizer and primary embryonic induction 813
- Competence and secondary induction 815

Syncytial Specification and Determination in *Drosophila* 815

51. Developmental Genetics

817-838

- Factors Controlling Development 818
- Changes in the Nucleus During Development (Nuclear Transplantation) 818
- Gene Regulation and Use of Markers During Development 819

- Gene regulation during early embryonic development 819
- Use of markers in developmental genetics 820

- Genetics of Development 821
 - Developmental genetics of *Drosophila* 822
 - Developmental genetics of higher plants 832

52. Immune System and Vaccines

839-869

- Natural Immunity and Acquired Immunity 840
 - Natural immunity 840
 - Acquired immunity 840

- Immune Responses 840
 - Lymphoid system (B-cells and T-cells) 840
 - Clonal selection theory 841
 - Complement system for antibodies 843

- Adjuvants and Immunosuppressants 844

- Structure of Immunoglobulins 844
 - Heavy and light chains forming Y-shaped structures 844
 - Three hypervariable regions (CDRs) forming antigen binding sites 845
 - Glycosylation of antibodies 846

- Genetics and Molecular Biology of the Production of Antibodies 846
 - Ig gene in germline and differentiated cells 847
 - V-regions through DNA splicing 847
 - V-C association for H-chain class switching 848
 - Allelic exclusion 848
 - Somatic hypermutations for fine tuning of antibody response 849

- T Cell Receptors and MHC Restriction 849
 - Types of T cells 850
 - T cell receptors and CD3 complex 850
 - MHC molecules and presentation of antigens to T cells 852

- T cell co-receptors (CD4 and CD8) 855
- Cytotoxic T cells and MHC restriction 856
- Helper T cells and T cells activation 857
- Two signals required for activation of helper T cell (e.g. interleukin-1 or IL-1 and B7+CD28) 858
- Helper T cells and interleukins (also called lymphokines or cytokines) 858
- MHC restriction and maturation/selection of developing T cells 860
- The peripheral tolerance paradox 861
- B cells, MHC molecules and immune response (*Ir*) genes 861
- Two subsets of CD4⁺ helper T lymphocytes (Th1 and Th2) 861
- Subsets of CD4⁺ helper cells (Th1 and Th2) and diseases 863
- Ig Superfamily and its members 863

- Tolerance of Autoimmunity and Autoimmune Diseases 864
 - Tolerance by clone elimination 865
 - Tolerance by clone retuning (anergy in B and T cells) 866
 - Tolerance by inhibiting clone effector functions 866
 - Effector molecules affecting the balance between tolerance and immunity 866

- Interferons 866

- Vaccine Development and Immunization 867
 - History and progress of vaccine development 867
 - Nature of vaccines 867

53. Genetics of Cancer : Proto-Oncogenes, Oncogenes and Tumour Suppressor Genes

870-878

- Viral Oncogenes (*v-onc* Genes) 870
- Isolated Oncogenes for Transfection Assay 873
- Activation of Proto-oncogenes by Mutations 873
 - Ras* proto-oncogenes 873
- Activation of Proto-oncogenes by Insertions, Translocations and Amplification 874
 - Insertions of retroviral sequences 874
 - Chromosomal translocations 874
 - Amplification of oncogenes in some tumours 875

- Transgenic mice for a study of oncogenic potential of *c-myc* gene 875

- Tumour Suppressor Genes or Anti-oncogenes 875
- Inherited Cancer Genes 876
- Cellular Functions of Oncoproteins 877
- Regulation of Gene Expression by Oncoproteins 877
- Signal Transduction by Oncoproteins (G Proteins) 878

54. Cell Death : Apoptosis

- Apoptosis in *Coenorhabditis elegans* 880
 - Specification of cells which should die 880
 - Actual death of specified cells 881
 - Engulfment of cell corpses 881
 - Degradation of cell corpses 881
- Cell Death in Mammals (Bcl-2 and ICE Proteins or Capsases) 881
- Apoptosis in *Drosophila* 883
- Apoptosis (Cell Death) in Other Cell Systems 883
 - Cell death during metamorphosis of moths 884
 - Cell death of vertebrate neurons deprived of growth factors 884
 - Cell death of thymocytes and mature T cells 884
 - Cell death in the regressing rat prostate 885
 - Programmed cell death in bacterial populations 885

879-89

- Apoptosis (Cell Death) in Cell Cultures 886
 - Genes that drive apoptosis 886
 - Genes that inhibit apoptosis 886
- Purpose of Cell Death 887
 - Cells that have no function 887
 - Cells that are produced in excess 887
 - Cells that develop improperly 888
 - Cells that have finished their function 888
 - Cells that are harmful 888
- Mechanism of Cell Death 888
- Apoptosis Targeted Therapies 890
 - Apoptosis in cells, which should not die 890
 - Absence of apoptosis in cells, which should die
 - Tumours due to absence of normally occurring inhibitors of growth 891

55. Pluripotent Stem Cells and Animal Cloning (Including Human Cloning)

892-90

- Stem Cells 893
 - Functions and origin of stem cells 893
 - Asymmetric division of stem cells 893
 - Types of stem cells 894
 - Adult stem cells vs embryonic / fetal stem cells 896
 - Migration of stem cells to injured tissues 897
 - Stem cell therapy to replace fetal neuron graft therapy 897
 - Human stem cells and biotechnology companies 898
 - Therapeutic cloning for embryonic stem cells (ESCs) : Ethical issues 898

- Animal Cloning 899
 - Nuclear transfer for cloning 899
 - Earliest efforts for animal cloning 900
 - Cloning from embryonic cells 901
 - Cloning from adult and fetal cells 901
 - Cloning from short-term cultured cells 902
 - Cloning from long-term cultured cells 903
 - Cloning for production of transgenic animals 903
 - Gene targeting for cloned transgenic animals 904
 - Cloning for conservation 905
 - Human cloning 905
 - Ethical issues and the risks associated with human cloning 905

References

907-9

Author Index

923-9

Subject Index

926-9