

CONTENTS

C O N T E N T S

PART I. CONCEPTS AND GENERAL METHODS	1
1. MOLECULAR BIOLOGY IN THE STUDY OF THE LIVING MATTER	3
<i>Zeno Garban</i>	
1.1. General considerations	3
1.2. Morphophysiologic diversity of living matter	5
1.3. Object of molecular biology. Interdisciplinarity	7
1.4. Historical references	9
1.5. Specific morphophysiological and topobiochemical elements of viruses	13
1.5.1. Synoptic view	13
1.5.2. Ultrastructure and topobiochemistry of viruses	14
1.5.2.1. Head	16
1.5.2.1.1. Core	17
1.5.2.1.2. Capsid.....	19
1.5.2.1.3. Peplos	19
1.5.2.2. Tail	20
1.5.2.2.1. Axial cylinder	20
1.5.2.2.1. Terminal plate	21
1.5.2.2.2. Terminal fibers.....	21
1.5.3. Vital cycle of viruses	22
1.5.3.1. Lytic cycle	22
1.5.3.2. Lysogenic cycle.....	23
1.6. Specific morphophysiological and topobiochemical elements of prokaryotes	24
1.6.1. Synoptic view	24
1.6.2. Ultrastructure and topobiochemistry of prokaryotic cells	25
1.6.2.1. Nucleoid	28
1.6.2.2. Cytoplasm	29
1.6.2.3. Intracellular elements	29
1.6.2.3.1. Ribosomes	30
1.6.2.3.2. Mesosomes	31

BIOLOGIE MOLECULAR : CONCEPTE, METODE, APLICĂ II

1.6.2.2.3. Plasmids	31
1.6.2.2.4. Storage droplets	32
1.6.2.2.5. Vacuoles	32
1.6.2.2.6. Photosynthetic apparatus	33
1.6.2.2.7. Other intracellular microbodies.....	33
1.6.2.4. Cell membrane	34
1.6.2.5. Flagella, pili, fimbriae	35
1.6.3. Cellular cycle in prokaryotes	36
1.7. Specific morphophysiological and topobiochemical elements of eukaryotes	36
1.7.1. Synoptic view	36
1.7.2. Ultrastructure and topobiochemistry of eukaryotic cells	37
1.7.2.1. Cell nucleus	38
1.7.2.2. Cytoplasm	40
1.7.2.3. Cell organelles	41
1.7.2.3.1. Mitochondria	41
1.7.2.3.2. Endoplasmic reticulum. Ribosomes.....	43
1.7.2.3.3. Golgi complex	46
1.7.2.3.4. Peroxisomes	46
1.7.2.3.5. Glyoxisomes.....	47
1.7.2.3.6. Lysosomes	47
1.7.2.3.7. Centrosome	48
1.7.2.3.8. Plastides	49
1.7.2.3.9. Vacuoles.....	50
1.7.2.4. Cytoskeleton	50
1.7.2.5. Cell membrane	51
1.7.2.5.1. Ultrastructure of membranes	52
1.7.2.5.2. Biologic role of membranes	54
1.7.2.6. Membrane barriers of cells	55
1.7.2.6.1. Transport systems – generalities	56
1.7.2.6.2. Microtransport systems	57
1.7.2.6.2.1. Passive transmembrane transport	57
1.7.2.6.2.2. Active transmembrane transport.....	63
1.7.2.6.3. Macrotransport systems	65
1.7.2.6.3.1. Endocytosis.....	65
1.7.2.6.3.2. Exocytosis.....	66
1.7.2.6.3.3. Specificity of macrotransport systems.....	67
1.7.2.7. Extracellular matrix	68

CONTENTS

1.7.2.7.1. Constitution of extracellular matrix	68
1.7.2.7.2. Interaction cell-extracellular matrix	70
1.7.3. Cell cycle	71
1.7.4. Tanatocytosis – a believable concept.....	73
2. INVESTIGATION OF ULTRASTRUCTURE AND BIOCONSTITUENTS	77
<i>Zeno Garban, Adina-Elena Avacovici, Gabriela Garban, Mirela Ahmadi</i>	
2.1. General methodology of investigations	77
2.1.1. Biological materials	77
2.1.1.1. Unimpaired animal organism	78
2.1.1.2. Organelles	78
2.1.1.3. Cells	80
2.1.1.4. Subcellular constituents	80
2.1.1.5. Viruses	81
2.1.1.6. Isolated enzymes	81
2.1.2. Microscopical procedures	81
2.1.2.1. General principles	82
2.1.2.2. Characteristics of microscopical procedures.....	83
2.1.2.3. Light microscopy	84
2.1.2.3.1. Light microscope	84
2.1.2.3.2. Specific techniques	85
2.1.2.4. Electron microscopy	87
2.1.2.4.1. Electron microscope.....	87
2.1.2.4.2. Specific techniques	88
2.1.2.5. Microscopic procedures in the study of nucleic acids of cells	91
2.1.2.5.1. Specific procedures of classic citochemistry	91
2.1.2.5.2. Specific procedures of molecular biology	93
2.1.2.5.3. Specific procedures of morphology and morphopathology	93
2.1.3. Biochemical methods	95
2.1.3.1. Metabolic analytical methods	97
2.1.3.2. Method of auxotrophe mutants	97
2.1.3.3. Isotopic methods	98
2.1.3.3.1. Use of stable isotopes	99
2.1.3.3.2. Use of radioactive isotopes	99
2.1.3.4. Chromatographic methods	101
2.1.3.4.1. Column chromatography.....	101

BIOLOGIE MOLECULAR : CONCEPTE, METODE, APLICĂ II

2.1.3.4.2. Paper chromatography	103
2.1.3.5. Spectrometric methods	105
2.1.3.5.1. Absorption spectrometry.....	108
2.1.3.5.2. Emission spectrometry.....	114
2.1.3.5.3. X-ray spectrometry.....	115
2.1.3.5.4. Fluorescence spectrometry.....	118
2.1.3.6. Stereochemical methods	120
2.1.3.7. Electrophoretical methods	123
2.1.4. Possibilities of integrated investigation	124
2.2. Ultrastructure of subcellular constituents	148
2.2.1. The role of cyto- and histochemical techniques	126
2.2.2. Fractionating of cell constituents	126
2.2.2.1. Principle of centrifugation	127
2.2.2.2. Types of centrifugation	129
2.2.2.2.1. Simple centrifugation	129
2.2.2.2.2. Zone centrifugation	130
2.2.2.2.3. Isopicnic centrifugation.....	130
2.2.2.3. Implications of cell fractionating	131
3. NUCLEIC ACIDS: CHEMICAL STRUCTURE AND BIOLOGICAL	
ACTIVITY	133
<i>Zeno Garban</i>	
3.1. General considerations	133
3.2. Structural components	135
3.2.1. Pentoses	135
3.2.2. Nucleobases	136
3.2.2.1. Pyrimidine nucleobases	137
3.2.2.2. Purine nucleobases	138
3.2.2.3. Nucleobases with reduced incidence	139
3.2.2.4. Tautomeria of nucleobases	139
3.2.3. Phosphoric acid	142
3.3. Structural precursors	143
3.3.1. Nucleosides	143
3.3.2. Nucleotides	146
3.3.2.1. Mononucleotides	146
3.3.2.2. Nucleotidic derivatives	148
3.3.2.2.1. Nucleoside polyphosphates	148
3.3.2.2.2. Polynucleotides	150
3.4. Nucleic acids and nucleoproteins	151

CONTENTS

3.4.1. General structural peculiarities	151
3.4.2. Intramolecular chemical bonds	152
3.5. Deoxyribonucleic acids	155
3.5.1. Chemical structure	155
3.5.1.1. Primary structure	155
3.5.1.2. Secondary structure.....	160
3.5.1.3. Tertiary structure	162
3.5.1.4. Quaternary structure.....	164
3.5.2. DNA families	165
3.5.2.1. DNA with dextrorotation	165
3.5.2.1.1. Stereochemical structural particularities	166
3.5.2.1.2. Descriptive elements of DNA families	169
3.5.2.2. DNA with senestrorotation	170
3.5.2.3. Intramolecular forces specific for DNA	172
3.5.2.4. Dextrorotation and senestrorotation at DNA	172
3.5.3. Topological peculiarities of DNA	173
3.6. Ribonucleic acids	175
3.6.1. Chemical structure	176
3.6.1.1. Primary structure	177
3.6.1.2. Secondary structure.....	177
3.6.1.3. Tertiary structure.....	179
3.6.2. RNA types	180
3.6.2.1. Messenger RNA	181
3.6.2.2. Transfer RNA	182
3.6.2.3. Ribosomal RNA	184
3.6.2.4. Small nuclear RNA.....	185
3.6.2.5. Macromolecular configurations in RNA	186
4. NUCLEIC ACIDS IN MODERN MOLECULAR BIOLOGY	187
<i>Zeno Garban</i>	
4.1. General considerations	187
4.2. Genes, nucleic acids, genetic genius	189
4.2.1. Synoptic frame	189
4.2.2. Biological information and nucleic acids	192
4.3. Relationship DNA-molecular filiation	195
4.3.1. Filiation levels	195
4.3.2. Living matter and DNA sequences	196
4.4. Electronic structures in nucleobases: peculiarities, importance	197

BIOLOGIE MOLECULAR : CONCEPTE, METODE, APLICA II

Bibliographical references (selective)	199
PART II. SPECIFIC METHODS IN MOLECULAR BIOLOGY	203
5. GENOME AND GENIC ORGANISATION OF LIVING MATTER	205
<i>Zeno Garban, Elisabeta-Mihaela Mitroi</i>	
5.1. General considerations	245
5.1.1. Genome in viruses	208
5.1.2. Genome in prokaryotes	210
5.1.3. Genome in eukaryotes.....	211
5.2. Genotype-phenotype-environment interaction	212
5.2.1. Concept of genotype	213
5.2.2. Concept of phenotype	214
5.2.3. The action of the environment	215
5.2.4. Genes and the genetic program - general coordinatives	216
5.3. Transmission of genetic information.....	217
5.3.1. Synoptic view	217
5.3.2. Process of replication.....	218
5.3.3. Process of transcription.....	222
5.3.4. Process of translation	225
5.3.4.1. Specificity of translation	225
5.3.4.2. Characteristics of the genetic code.....	227
5.3.4.3. Translation apparatus	232
5.3.4.4. Activation of amino acids	233
5.3.4.5. Biosynthesis of proteins – steps	235
5.3.4.6. Energetics and the regulation of protein biosynthesis.....	239
6. INVESTIGATION OF NUCLEIC ACIDS IN MOLECULAR BIOLOGY... 241	
<i>Zeno Garban, Gabriela Garban, Sorina Mihacea, Adina-Elena Avacovici</i>	
6.1. General considerations	241
6.2. Methods for determination the sequences of nucleic acids	243
6.2.1. Synoptic view	243
6.2.2. Maxam-Gilbert method	244
6.2.3. Sanger method	245
6.2.4. General applications	247
6.3. Methods of hybridisation of nucleic acids chains	247
6.3.1. Synoptic view	247
6.3.2. Types of hybridisation	248
6.3.3. Dissociation and association of nucleic acids.....	249

CONTENTS

6.3.3.1. Dissociation of nucleic acids	250
6.3.3.1.1. Hyperchromic effect.....	250
6.3.3.1.2. Melting temperature	251
6.3.3.2. Association of nucleic acids	252
6.3.3.2.1. Velocity of association reaction	253
6.3.3.2.2. Kinetics of association reaction	255
6.3.4. Investigation of the dissociation and association reactions	259
6.3.5. General applications	260
6.4. Methods of detection by molecular sondes	260
6.4.1. Synoptic view	260
6.4.2. Radiomarked molecular sondes	261
6.4.3. Chemiomarked molecular sondes	262
6.4.3.1. Directly marked molecular sondes	262
6.4.3.2. Indirectly marked molecular sondes	263
6.5. Methods of investigation the molecular fragment of nucleic acids ..	263
6.5.1. Synoptic view	263
6.5.2. Southern method	264
6.5.3. Northern method	265
6.5.4. General applications	265
6.6. Method of amplification by polymerase chain reaction (PCR)	266
6.6.1. Synoptic view	266
6.6.2. Methodological principles	266
6.6.2.1. Particularities of the amplification cycle by PCR.....	268
6.6.2.2. DNA investigation by the PCR amplification method	270
6.6.3. Specific procedures of the PCR method	271
6.6.3.1. End-point PCR procedure (EP-PCR).....	271
6.6.3.2. Real time quantitative PCR procedure (Q-PCR)	271
6.6.3.3. Reverse-transcription PCR procedure (RT-PCR)	273
6.6.4. Applications of the polymorphism chain reaction (PCR)	276
6.6.4.1. Applications of end-point PCR (EP-PCR).....	276
6.6.4.2. Applications of real time quantitative PCR (Q-PCR)	277
6.6.4.3. Applications of reverse-transcription PCR techniques (RT-PCR)	277
6.7. Method of evaluation of the restriction fragment length polymorphism (RFLP)	278
6.7.1. Synoptic view	278
6.7.2. Methodological principles	278
6.7.3. Applications of the methods of evaluation of RFLP	280
6.7.3.1. Reverse genetics	281
6.7.3.2. DNA fingerprint	283

6.8. Method to evaluate the amplified fragment length polymorphism (AFLP)	284
6.8.1. Synoptic view	284
6.8.2. Methodological principles	285
6.8.3. Applications of the AFLP evaluation method	286
6.9. Genic recombination – synopsis	287
6.9.1. Transfer of genes by means of plasmids	287
6.9.1.1. Procedure of genomic DNA usage	289
6.9.1.2. Procedure of messenger RNA usage	290
7. NATURAL GENIC RECOMBINATION	291
<i>Gabriela Garban, Adina-Elena Avacovici, Ariana-Bianca Velciov, Zeno Garban</i>	
7.1. General considerations	291
7.2. Genic recombination in viruses	292
7.3. Genic recombination in prokaryotes	293
7.3.1. Transformation.....	294
7.3.2. Conjugation.....	297
7.3.3. Sexduction	299
7.3.4. Transduction	300
7.4. Genic recombination in eukaryotes	302
8. TECHNOLOGICALLY MEDIATED GENIC RECOMBINATION	305
<i>Zeno Garban</i>	
8.1. General considerations	305
8.2. Operating means for recombinant DNA	306
8.2.1. Restriction and modification enzymes.....	308
8.2.1.1. Restriction enzymes	312
8.2.1.2. Modification enzymes.....	364
8.2.2. Enzymes of DNA polymerases group.....	313
8.2.2.1. DNA-polymerases - DNA dependent	313
8.2.2.2. DNA polymerases - RNA dependent.....	314
8.2.3. Enzymes of the DNA ligases group.....	314
8.3. Strategies for the obtainment of recombinant DNA	316
8.3.1. General principles	316
8.3.2. Concept of vector.....	316
8.3.2.1. Plasmids	317
8.3.2.2. Bacteriophages	319
8.3.2.3. Cosmides.....	320
8.3.3. Concept of passenger	321
8.3.4. Recombinant DNA molecules formation	322

CONTENTS

8.3.5. Cloning of recombinant DNA.....	323
8.4. Genic Recombination at the interface with xenobiochemistry	326
8.4.1. Comparative data – xenobiochemical attributes	326
8.4.2. Genic recombination and protein synthesis	328
Bibliographical references (selective)	331
PART III. APPLICATIONS OF MOLECULAR BIOLOGY	335
9. BIOTECHNOLOGICAL APPLICATIONS	337
<i>Zeno Garban, Sorina Mihacea, George-Daniel Ghibu, Teodor Vintil , Elisabeta-Mihaela Mitroi</i>	
9.1. General considerations	337
9.2. Specificity of applicative domains	337
9.3. Applications of pharmaceutical interest	338
9.3.1. Obtainment of hormones	339
9.3.1.1. Somatostatine.....	339
9.3.1.2. Insulin	340
9.3.1.3. Somatotrope hormone.....	341
9.3.2. Obtainment of mediators for immune response	342
9.3.2.1. Interferons	342
9.3.2.2. Interleukins	345
9.4. Application of nutritional interest.....	346
9.4.1. Enzymes with attributes of food additives.....	347
9.4.1.1. Enzymes in general biochemistry	347
9.4.1.2. Enzymes in food biotechnology.....	352
9.4.2. Obtainment of enzymatic microbiologic additives	354
9.4.3. Obtainment of enzymatic biochemical additives	356
9.4.4. Obtainment of enzymatic compounds by methods specific for molecular biology	356
9.4.5. Investigations on enzymatic systems in situ	358
9.5. Applications in plant and animal biology	359
9.5.1. Biodiversity – conceptual aspects	359
9.5.2. Applications referring to plants	361
9.5.3. Applications referring to animals	364
9.5.4. Chimeras in biology	367
9.5.4.1. General data	367
9.5.4.2. Chimeras in vegetal kingdom	368
9.5.4.3. Chimeras in animal kingdom.....	368
9.5.4.3.1. Intraspecific chimeras	369
9.5.4.3.1. Interspecific chimeras	371
9.5.4.4. Animal chimeras in scientific research	371

BIOLOGIE MOLECULAR : CONCEPTE, METODEDE, APLICA II

9.6. Applications of agrobiological interest	373
9.6.1. Synoptic view	373
9.6.2. Transgenesis: from concepts to applications	374
9.6.2.1. Methods for genes transfer	374
9.6.2.2. Phases of transgenesis	375
9.6.3. Genetically modified organisms	376
9.6.3.1. Aspects in agriculture	376
9.6.3.2. Aspects in zooculture	378
9.6.4. Transgenicity and environment	379
9.6.5. Problems of sustainable agriculture	381
9.7. Cisgenesis and transgenesis	382
9.7.1. Connected aspects	382
9.7.2. Specificity of processes	383
9.7.3. Operation ways – phases	383
9.8. Biotechnologies – synoptic frame	384
9.8.1. Interdisciplinary aspects	384
9.8.2. Interconnections of biotechnologies	385
9.8.2.1. Biological processes	385
9.8.2.2. Technological equipments	385
9.8.2.3. Industrial processing	386
10. BIOMEDICAL APPLICATIONS	387
<i>Gabriela Garban, Roxana-Daniela Vintil , Dan-Bogdan Navolan, George-Daniel Ghibu, Zeno Garban</i>	
10.1. General considerations	387
10.2. Exploration of filiation levels	387
10.3. Diagnostic problems in hereditary pathology	388
10.3.1. Prenatal diagnostics	389
10.3.2. Postnatal diagnostics	390
10.3.3. Investigations in obstetrics and gynecology	391
10.4. Totipotent and pluripotent stem cells in moderne medicine	397
10.4.1. Synoptic frame	397
10.4.2. Pluripotent stem cell types	398
10.4.2.1. Pluripotent embryonic stem cells	398
10.4.2.2. Pluripotent adult stem cells	399
10.4.3. Applications of pluripotent stem cells	400
10.4.3.1. Applications of pluripotent embryonic stem cells	400
10.4.3.2. Applications of pluripotent adult stem cells	401
10.5. Xenotranplants: present and future	403
10.5.1. Synoptic frame	403

CONTENTS

10.5.2. Xenotransplant rejection	403
10.5.3. Modern attempt to achieve xenotransplantation	404
10.5.3.1. General ways to achieve tolerance to xenotransplant	405
10.5.3.2. Additional ways to analyze tolerance to xenotransplant	406
10.5.4. Perspectives of xenotransplants	407
10.6. Infection detection	408
10.7. Obtainment of vaccines	408
10.8. Investigations in forensic medicine	410
10.9. Mapping of human genome	412
11. CHRONOBIOCHEMICAL APPLICATIONS	415
<i>Zeno Garban, Gabriela Garban, Adina-Elena Avacovici</i>	
11.1. General considerations	415
11.2. Biorhythms and molecular biology	416
11.2.1. Periodicity of biorhythms	416
11.2.2. Conditioning of biorhythms	417
11.3. Main types of biorhythms	418
11.3.1. Ultradiene biorhythms	418
11.3.2. Circadian biorhythms	418
11.3.3. Infradiene biorhythms	420
11.4. Chronobiochemical parameters specific for biologic rhythms	422
11.5. Autonomy and synchronisation of biorhythms	424
11.5.1. Autonomy of biorhythms	424
11.5.2. Synchronisation of biorhythms	425
11.6. Implications of molecular biology and chronobiochemistry in aging ..	426
12. APPLICATIONS IN THE STUDY OF XENOBIOTICS	429
<i>Zeno Garban</i>	
12.1. General considerations	429
12.2. Xenobiotics of nutritional interest	431
12.2.1. Food additives	432
12.2.2. Chemical food pollutants	435
12.2.3. Adverse reaction with xenobiotic specificity	438
12.3. Xenobiotics of pharmaceutical interest	441
12.3.1. Chemotherapeutic drugs	441
12.3.2. Cytostatic chemotherapics	442
12.4. Xenobiotics with nutritional interest and chemical carcinogenesis	442

BIOLOGIE MOLECULAR : CONCEPTE, METODE, APLICA II

12.4.1. Synoptic view	442
12.4.2. Deoxyribonucleic acid in chemical carcinogenesis	443
12.4.3. Interaction of some polycyclic organic compounds and some metal ions with DNA	445
12.4.4. DNA adducts with polycyclic organic compounds and metal ions	451
12.5 Xenobiotics of pharmaceutical interest used in cytostatic chemotherapy	456
12.5.1. Synoptic view	456
12.5.2. Deoxyribonucleic acid in cytostatic chemotherapy	457
12.5.3. Interaction of some chemotherapeutics with DNA	459
12.5.4. Complexes DNA-cytostatics	460
Bibliographical references (selective)	467
Addenda.....	475
Minimal glossary	481
Retrospectives.....	495
Subject index	497
Remember.....	503