

Contents

<i>Preface</i>	xvii
<i>Acknowledgments</i>	xxi
<i>About the Authors</i>	xxiii

PART I The Science of Virology 1

1 Foundations 2

Luria's Credo 3

Why We Study Viruses 3

- Viruses Are Everywhere 3
- Viruses Can Cause Human Disease 3
- Viruses Infect All Living Things 3
- Viruses Can Be Beneficial 4
- Viruses Can Cross Species Boundaries 4
- Viruses "R" Us 4
- Viruses Are Unique Tools To Study Biology 5

Virus Prehistory 6

- Viral Infections in Antiquity 6
- The First Vaccines 7
- Microorganisms as Pathogenic Agents 9

Discovery of Viruses 10

The Definitive Properties of Viruses 12

- The Structural Simplicity of Virus Particles 12
- The Intracellular Parasitism of Viruses 14

Viruses Defined 17

Cataloging Animal Viruses 17

- The Classical System 17
- Classification by Genome Type: the Baltimore System 20

A Common Strategy for Viral Propagation	21
Perspectives	21
References	23

2 The Infectious Cycle 24

Introduction	25
The Infectious Cycle	25
The Cell	25
The Architecture of Cell Surfaces	27
The Extracellular Matrix: Components and Biological Importance	27
Properties of the Plasma Membrane	29
Cell Membrane Proteins	30
Entering Cells	31
Making Viral RNA	31
Making Viral Proteins	31
Making Viral Genomes	31
Forming Progeny Virus Particles	31
Viral Pathogenesis	32
Overcoming Host Defenses	32
Cultivation of Viruses	32
Cell Culture	32
Embryonated Eggs	35
Laboratory Animals	35
Assay of Viruses	36
Measurement of Infectious Units	36
Efficiency of Plating	39
Measurement of Virus Particles and Their Components	39
Viral Reproduction: the Burst Concept	46
The One-Step Growth Cycle	46
Initial Concept	46
One-Step Growth Analysis: a Valuable Tool for Studying Animal Viruses	49
Systems Biology	50
Perspectives	51
References	52

PART II

Molecular Biology 53

3 Genomes and Genetics 54

Introduction	55
Genome Principles and the Baltimore System	55

Structure and Complexity of Viral Genomes	55
DNA Genomes	56
RNA Genomes	58
What Do Viral Genomes Look Like?	59
Coding Strategies	60
What Can Viral Sequences Tell Us?	60
The Origin of Viral Genomes	61
The "Big and Small" of Viral Genomes: Does Size Matter?	65
Genetic Analysis of Viruses	65
Classical Genetic Methods	66
Engineering Mutations into Viral Genomes	67
Engineering Viral Genomes: Viral Vectors	73
Perspectives	78
References	79

4 **Structure** 80

Introduction	81
Functions of the Virion	81
Nomenclature	82
Methods for Studying Virus Structure	83
Building a Protective Coat	86
Helical Structures	86
Capsids with Icosahedral Symmetry	89
Other Capsid Architectures	102
Packaging the Nucleic Acid Genome	104
Direct Contact of the Genome with a Protein Shell	104
Packaging by Specialized Viral Proteins	105
Packaging by Cellular Proteins	105
Viruses with Envelopes	106
Viral Envelope Components	106
Simple Enveloped Viruses: Direct Contact of External Proteins with the Capsid or Nucleocapsid	109
Enveloped Viruses with an Additional Protein Layer	109
Large Viruses with Multiple Structural Elements	111
Bacteriophage T4	111
Herpesviruses	112
Poxviruses	113
Giant Viruses	114
Other Components of Virions	116
Enzymes	116
Other Viral Proteins	116
Nongenomic Viral Nucleic Acid	117
Cellular Macromolecules	117
Perspectives	119
References	119

5	Attachment and Entry	122
	Introduction	123
	Attachment of Virus Particles to Cells	123
	General Principles	123
	Identification of Receptors for Virus Particles	124
	Virus-Receptor Interactions	126
	Entry into Cells	132
	Uncoating at the Plasma Membrane	132
	Uncoating during Endocytosis	135
	Membrane Fusion	137
	Movement of Viral and Subviral Particles within Cells	147
	Virus-Induced Signaling via Cell Receptors	148
	Import of Viral Genomes into the Nucleus	148
	Nuclear Localization Signals	149
	The Nuclear Pore Complex	149
	The Nuclear Import Pathway	150
	Import of Influenza Virus Ribonucleoprotein	151
	Import of DNA Genomes	151
	Import of Retroviral Genomes	151
	Perspectives	153
	References	154
6	Synthesis of RNA from RNA Templates	156
	Introduction	157
	The Nature of the RNA Template	157
	Secondary Structures in Viral RNA	157
	Naked or Nucleocapsid RNA	158
	The RNA Synthesis Machinery	159
	Identification of RNA-Dependent RNA Polymerases	159
	Sequence Relationships among RNA Polymerases	161
	Three-Dimensional Structure of RNA-Dependent RNA Polymerases	161
	Mechanisms of RNA Synthesis	164
	Initiation	164
	Capping	168
	Elongation	168
	Template Specificity	169
	Unwinding the RNA Template	169
	Role of Cellular Proteins	170
	Paradigms for Viral RNA Synthesis	170
	(+) Strand RNA	171
	Synthesis of Nested Subgenomic mRNAs	172
	(-) Strand RNA	173
	Ambisense RNA	174

Double-Stranded RNA 175

Unique Mechanisms of mRNA and Genome Synthesis of
Hepatitis Delta Satellite Virus 176

Why Are (–) and (+) Strands Made in Unequal Quantities? 177

Do Ribosomes and RNA Polymerases Collide? 179

Cellular Sites of Viral RNA Synthesis 179

Origins of Diversity in RNA Virus Genomes 182

Misincorporation of Nucleotides 182

Segment Reassortment and RNA Recombination 183

RNA Editing 185

Perspectives 185

References 185

7 Reverse Transcription and Integration 188

Retroviral Reverse Transcription 189

Discovery 189

Impact 189

The Process of Reverse Transcription 189

General Properties and Structure of Retroviral Reverse Transcriptases 198

Other Examples of Reverse Transcription 202

Retroviral DNA Integration Is a Unique Process 204

The Pathway of Integration: Integrase-Catalyzed Steps 205

Integrase Structure and Mechanism 210

Hepadnaviral Reverse Transcription 214

A DNA Virus with Reverse Transcriptase 214

The Process of Reverse Transcription 216

Perspectives 221

References 222

8 Synthesis of RNA from DNA Templates 224

Introduction 225

Properties of Cellular RNA Polymerases That Transcribe Viral DNA 225

Some Viral Genomes Must Be Converted to Templates Suitable
for Transcription 226

Transcription by RNA Polymerase II 228

Regulation of RNA Polymerase II Transcription 228

Common Properties of Proteins That Regulate Transcription 234

The Cellular Machinery Alone Can Transcribe Viral DNA Templates 235

Viral Proteins That Govern Transcription of Viral DNA Templates 237

Patterns of Regulation 237

The Human Immunodeficiency Virus Type 1 Tat Protein Autoregulates
Transcription 237

The Transcriptional Cascades of DNA Viruses	245
Entry into One of Two Alternative Transcriptional Programs	254
Transcription of Viral Genes by RNA Polymerase III	257
The VA-RNA I Promoter	257
Regulation of VA-RNA Gene Transcription	259
Inhibition of the Cellular Transcriptional Machinery	259
Unusual Functions of Cellular Transcription Components	260
A Viral DNA-Dependent RNA Polymerase	260
Perspectives	262
References	263

9 Replication of DNA Genomes 266

Introduction	267
DNA Synthesis by the Cellular Replication Machinery	269
Eukaryotic Replicons	269
Cellular Replication Proteins	270
Mechanisms of Viral DNA Synthesis	271
Lessons from Simian Virus 40	271
Replication of Other Viral DNA Genomes	275
Properties of Viral Replication Origins	278
Recognition of Viral Replication Origins	280
Viral DNA Synthesis Machines	286
Resolution and Processing of Viral Replication Products	287
Exponential Accumulation of Viral Genomes	288
Viral Proteins Can Induce Synthesis of Cellular Replication Proteins	288
Synthesis of Viral Replication Machines and Accessory Enzymes	290
Viral DNA Replication Independent of Cellular Proteins	291
Delayed Synthesis of Structural Proteins Prevents Premature	
Packaging of DNA Templates	291
Inhibition of Cellular DNA Synthesis	291
Viral DNAs Are Synthesized in Specialized Intracellular Compartments	292
Limited Replication of Viral DNA Genomes	296
Integrated Parvoviral DNA Can Replicate as Part of the Cellular Genome	296
Different Viral Origins Regulate Replication of Epstein-Barr Virus	297
Limited and Amplifying Replication from a Single Origin:	
the Papillomaviruses	299
Origins of Genetic Diversity in DNA Viruses	301
Fidelity of Replication by Viral DNA Polymerases	301
Inhibition of Repair of Double-Strand Breaks in DNA	303
Recombination of Viral Genomes	304
Perspectives	307
References	307

10 Processing of Viral Pre-mRNA 310

Introduction 311

Covalent Modification during Viral Pre-mRNA Processing 312

Capping the 5' Ends of Viral mRNA 312

Synthesis of 3' Poly(A) Segments of Viral mRNA 315

Splicing of Viral Pre-mRNA 317

Alternative Processing of Viral Pre-mRNA 322

Editing of Viral mRNAs 325

Export of RNAs from the Nucleus 327

The Cellular Export Machinery 327

Export of Viral mRNA 327

Posttranscriptional Regulation of Viral or Cellular Gene Expression by Viral Proteins 330

Temporal Control of Viral Gene Expression 330

Viral Proteins Can Inhibit Cellular mRNA Production 333

Regulation of Turnover of Viral and Cellular mRNAs in the Cytoplasm 335

Regulation of mRNA Stability by Viral Proteins 336

mRNA Stabilization Can Facilitate Transformation 338

Production and Function of Small RNAs That Inhibit Gene Expression 338

Small Interfering RNAs, Micro-RNAs, and Their Synthesis 338

Viral Micro-RNAs 342

Viral Gene Products That Block RNA Interference 345

Perspectives 345

References 346

11 Protein Synthesis 348

Introduction 349

Mechanisms of Eukaryotic Protein Synthesis 349

General Structure of Eukaryotic mRNA 349

The Translation Machinery 350

Initiation 351

Elongation and Termination 360

The Diversity of Viral Translation Strategies 362

Polyprotein Synthesis 363

Leaky Scanning 365

Reinitiation 366

Suppression of Termination 366

Ribosomal Frameshifting 368

Bicistronic mRNAs 368

Regulation of Translation during Viral Infection 368

Inhibition of Translation Initiation after Viral Infection 369

Regulation of eIF4F 372

- Regulation of Poly (A)-Binding Protein Activity 376
- Regulation of eIF3 376
- Interfering with RNA 376
- Stress-Associated RNA Granules 377

Perspectives 377

References 379

12 Intracellular Trafficking 380

Introduction 381

Assembly within the Nucleus 382

- Import of Viral Proteins for Assembly 383

Assembly at the Plasma Membrane 384

- Transport of Viral Membrane Proteins to the Plasma Membrane 386
- Sorting of Viral Proteins in Polarized Cells 401
- Disruption of the Secretory Pathway in Virus-Infected Cells 404
- Signal Sequence-Independent Transport of Viral Proteins to the Plasma Membrane 406

Interactions with Internal Cellular Membranes 409

- Localization of Viral Proteins to Compartments of the Secretory Pathway 410
- Localization of Viral Proteins to the Nuclear Membrane 411

Transport of Viral Genomes to Assembly Sites 411

- Transport of Genomic and Pregenomic RNA from the Nucleus to the Cytoplasm 411
- Transport of Genomes from the Cytoplasm to the Plasma Membrane 411

Perspectives 413

References 414

13 Assembly, Exit, and Maturation 416

Introduction 417

Methods of Studying Virus Assembly and Egress 418

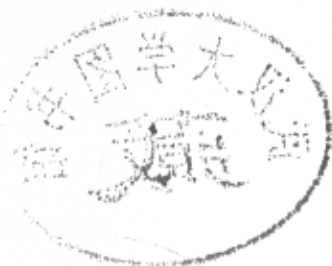
- Structural Studies of Virus Particles 418
- Visualization of Assembly and Exit by Microscopy 418
- Biochemical and Genetic Analyses of Assembly Intermediates 418
- Methods Based on Recombinant DNA Technology 421

Assembly of Protein Shells 421

- Formation of Structural Units 421
- Capsid and Nucleocapsid Assembly 423
- Self-Assembly and Assisted Assembly Reactions 425

Selective Packaging of the Viral Genome and Other Components of Virus Particles 430

- Concerted or Sequential Assembly 430
- Recognition and Packaging of the Nucleic Acid Genome 431
- Incorporation of Enzymes and Other Nonstructural Proteins 438



Acquisition of an Envelope 439

Sequential Assembly of Internal Components and Budding
from a Cellular Membrane 439

Coordination of the Assembly of Internal Structures
with Acquisition of the Envelope 440

Release of Virus Particles 441

Assembly and Budding at the Plasma Membrane 441

Assembly at Internal Membranes: the Problem of Exocytosis 444

Release of Nonenveloped Viruses 450

Maturation of Progeny Virus Particles 450

Proteolytic Processing of Structural Proteins 450

Other Maturation Reactions 456

Cell-to-Cell Spread 457**Perspectives 460****References 460****14 The Infected Cell 464****Introduction 465****Signal Transduction 465**

Signaling Pathways 465

Signaling in Virus-Infected Cells 466

Gene Expression 470

Inhibition of Cellular Gene Expression 470

Differential Regulation of Cellular Gene Expression 474

Metabolism 477

Methods To Study Metabolism 477

Glucose Metabolism 479

The Citric Acid Cycle 483

Electron Transport and Oxidative Phosphorylation 484

Lipid Metabolism 486

Remodeling of Cellular Organelles 491

The Nucleus 491

The Cytoplasm 495

Perspectives 498**References 500****APPENDIX Structure, Genome Organization, and Infectious Cycles 501****Glossary 537****Index 543**