

BRIEF CONTENTS

SECTION I: INTRODUCTION TO VIROLOGY

- 1. Introduction to Virology** 2
Nicholas H. Acheson, McGill University
Christopher D. Richardson, Dalhousie University
- 2. Virus Structure and Assembly** 19
Stephen C. Harrison, Harvard University
- 3. Virus Classification: The World of Viruses** 32
Nicholas H. Acheson, McGill University
Christopher D. Richardson, Dalhousie University
- 4. Virus Entry** 47
Ari Helenius, Swiss Federal Institute of Technology, Zurich

SECTION II: VIRUSES OF BACTERIA AND ARCHAEA

- 5. Single-Stranded RNA Bacteriophages** 60
Jan van Duin, University of Leiden
Karthik Chamakura, Armata Pharmaceuticals, Inc., Los Angeles
Ryland Young, Texas A&M University
- 6. Microviruses** 74
Bentley A. Fane, University of Arizona
Aaron P. Roznowski, University of Arizona
- 7. Bacteriophage T7** 84
William C. Summers, Yale University
Ian J. Molineux, University of Texas, Austin
- 8. Bacteriophage T4** 94
Deborah M. Hinton, National Institutes of Health, Bethesda
Eric S. Miller, North Carolina State University
- 9. Bacteriophage Lambda** 110
Michael Feiss, University of Iowa
- 10. Viruses of Archaea** 123
David Prangishvili, Institut Pasteur, Paris
Mart Krupovic, Institut Pasteur, Paris

SECTION III: POSITIVE-STRAND RNA ANIMAL VIRUSES

- 11. Picornaviruses** 140
Bert L. Semler, University of California, Irvine
- 12. Flaviviruses** 152
Richard Kuhn, Purdue University
Shelton Bradrick, Trudeau Institute, New York
- 13. Hepaciviruses** 164
John Lok Man Law, Memorial University of Newfoundland
Michael Houghton, University of Alberta
- 14. Togaviruses and Rubella Virus** 178
Anil Kumar, University of Saskatchewan
Milton Schlesinger, Washington University, St. Louis
Sondra Schlesinger, Washington University, St. Louis
Tom C. Hobman, University of Alberta
- 15. Coronaviruses** 192
Marc Desforges, Ste. Justine Hospital, Université de Montréal
Pierre Talbot, Institut Armand-Frappier
Mark Denison, Vanderbilt University

SECTION IV: NEGATIVE-STRAND AND DOUBLE-STRANDED RNA ANIMAL VIRUSES

- 16. Paramyxoviruses and Pneumoviruses** 210
Nicholas H. Acheson, McGill University
Daniel Kolakofsky, University of Geneva
Laurent Roux, University of Geneva
Christopher D. Richardson, Dalhousie University
- 17. Rhabdoviruses** 226
Valery Grdzelisvili, University of North Carolina, Charlotte
Cassandra A. Catacalos, University of North Carolina, Charlotte
- 18. Filoviruses** 237
Heinz Feldmann, Rocky Mountain Laboratories, Montana
Hans-Dieter Klenk, University of Marburg
Nicholas H. Acheson, McGill University
Angela Rasmussen, University of Saskatchewan

19. Bunyaviruses 251

Richard M. Elliott, University of Glasgow
Lev Levanov, University of Helsinki
Alexander Plyusnin, University of Helsinki

20. Influenza Viruses 262

Dalius J. Briedis, McGill University
Alyson Kelvin, University of Calgary

21. Reoviruses 278

Kristen M. Ogden, Vanderbilt University
Terence S. Dermody, University of Pittsburgh School of Medicine

SECTION V: SMALL DNA ANIMAL VIRUSES

22. Parvoviruses 292

Peter Beard, École Polytechnique Fédérale de Lausanne
Sarah Wootton, University of Guelph

23. Polyomaviruses 302

Nicholas H. Acheson, McGill University
James A. DeCaprio, Dana-Farber Cancer Institute, Harvard University

24. Papillomaviruses 318

Greg Matlaszewski, McGill University
Lawrence Banks, International Centre for Genetic Engineering and Biotechnology, Trieste
Miranda Thomas, International Centre for Genetic Engineering and Biotechnology, Trieste

SECTION VI: LARGE DNA ANIMAL VIRUSES

25. Adenoviruses 330

Philip Branton, McGill University
Richard C. Marcellus, McGill University
Luca D. Bertzbach, Leibniz Institute of Virology, Hamburg
Thomas Dobner, Leibniz Institute of Virology, Hamburg

26. Herpesviruses 344

Bernard Roizman, University of Chicago
Gabriella Campadelli-Fiume, University of Bologna
Richard Longnecker, Northwestern University
Bruce Banfield, Queens University
Craig McCormick, Dalhousie University

27. Poxviruses 366

Richard Condit, University of Florida
Matthew D. Gresseth, Medical University of South Carolina
Paula Traktman, Medical University of South Carolina

SECTION VII: VIRUSES WITH A REVERSE TRANSCRIPTASE

28. Retroviruses 382

Alan Cochrane, University of Toronto

29. Human Immunodeficiency Virus 394

Alan Cochrane, University of Toronto

30. Hepadnaviruses 406

Christopher D. Richardson, Dalhousie University
William Addison, University of Alberta
D. Lorne Tyrrell, University of Alberta

SECTION VIII: VIROIDS AND PRIONS

31. Viroids and Hepatitis Delta Virus 420

Jean-Pierre Perreault, Université de Sherbrooke
Martin Pelchat, University of Ottawa
Charith Raj Adkar-Purusbothama, Université de Sherbrooke

32. Prions 431

Dalius J. Briedis, McGill University
David Westaway, University of California, San Francisco

SECTION IX: VIRUSES OF PLANTS, ALGAE, AND INVERTEBRATES

33. Cucumber Mosaic Virus 444

Marilyn J. Roossinck, Pennsylvania State College of Agricultural Sciences

34. Viruses of Algae and Mimivirus, a Giant Virus 457

Michael J. Allen, University of Exeter
William H. Wilson, Marine Biological Association, Plymouth
John A. Duffy, University of Exeter

35. Baculoviruses 478

Eric Carstens, Queens University
Robert L. Harrison, U.S. Department of Agriculture, Beltsville, Maryland

36. Viruses of Invertebrates 489*Peter Krell, University of Guelph***SECTION X: HOST DEFENSES AGAINST VIRUS INFECTION**

37. Innate Immune Responses Against Virus Infection 506*Karen Mossman, McMaster University**John Hiscott, Istituto Pasteur-Fondazione Cenci Bolognetti, Rome**Alessandra Zevini, Istituto Pasteur-Fondazione Cenci**Bolognetti, Rome***38. Adaptive Immune Responses to Virus Infection 527***Malcolm G. Baines, McGill University**Karen Mossman, McMaster University**Naglaa Shoukry, University of Montreal***SECTION XI: MEDICAL APPLICATIONS OF VIROLOGY**

39. Antiviral Vaccines 542*Brian Ward, McGill University**Hilary E. Hendin, McGill University***40. Antiviral Chemotherapy 562***Donald M. Coen, Harvard Medical School***41. Oncolytic Viruses 578***Vishnupriyan Kumar, Dalhousie University**Liang-Tzung Lin, Taipei Medical University**Shashi Gujar, Dalhousie University***42. Virus-Mediated Gene Therapy 586***Richard Peluso, Renovacor, Philadelphia**Christopher D. Richardson, Dalhousie University*

CONTENTS

SECTION I: INTRODUCTION TO VIROLOGY

1. Introduction to Virology 2

THE NATURE OF VIRUSES 3

Viruses consist of a nucleic acid genome packaged in a protein coat 3

Viruses are dependent on living cells for their replication 3

Virus particles break down and release their genomes inside the cell 4

Virus genomes are either RNA or DNA, but not both 4

WHY STUDY VIRUSES? 4

Viruses are important disease-causing agents 4

Viruses can infect all forms of life 5

Viruses are the most abundant form of life on Earth 5

The study of viruses has led to numerous discoveries in molecular and cell biology 6

A BRIEF HISTORY OF VIROLOGY:

THE STUDY OF VIRUSES 6

The scientific study of viruses is very recent 6

Viruses were first distinguished from other microorganisms by filtration 6

The crystallization of tobacco mosaic virus challenged conventional notions about genes and the nature of living organisms 8

The “phage group” stimulated studies of bacteriophages and helped establish the field of molecular biology 8

Study of tumor viruses led to discoveries in molecular biology and understanding of the nature of cancer 9

DETECTION AND TITRATION OF VIRUSES 10

Most viruses were first detected and studied by infection of intact organisms 10

The plaque assay arose from work with bacteriophages 10

Eukaryotic cells cultured in vitro have been adapted for plaque assays 10

Hemagglutination is a convenient and rapid assay for many viruses 11

Virus particles can be seen and counted by electron microscopy 12

Virus genome copy equivalents can be determined by quantitative polymerase chain reaction (qPCR) 12

The ratio of physical virus particles to infectious particles can be much greater than 1 12

THE VIRUS REPLICATION

CYCLE: AN OVERVIEW 13

The single-cycle virus replication experiment 13

An example of a virus replication cycle: mouse polyomavirus 13

Analysis of viral macromolecules reveals the detailed pathways of virus replication 13

STEPS IN THE VIRUS REPLICATION CYCLE 14

1. Virions bind to receptors on the cell surface 14

2. The virion (or the viral genome) enters the cell 15

3. Early viral genes are expressed: the Baltimore classification of viruses 15

The seven groups in the Baltimore classification system 16

4. Early viral proteins direct replication of viral genomes 17

5. Late messenger RNAs are made from newly replicated genomes 17

6. Late viral proteins package viral genomes and assemble virions 17

7. Progeny virions are released from the host cell 17

2. Virus Structure and Assembly 19

BASIC CONCEPTS OF VIRUS STRUCTURE 19

Virus structure is studied by electron microscopy and X-ray diffraction 20

Many viruses come in simple, symmetrical packages 20

CAPSIDS WITH ICOSAHERAL SYMMETRY 22

Some examples of virions with icosahedral symmetry 22

The concept of quasi-equivalence 23

Larger viruses come in more complex packages 24

CAPSIDS WITH HELICAL SYMMETRY 26

VIRAL ENVELOPES 26

Viral envelopes are made from lipid bilayer membranes 26

Viral glycoproteins are inserted into the lipid membrane to form the envelope 27

PACKAGING OF GENOMES AND VIRION ASSEMBLY 28

Multiple modes of capsid assembly 28

Specific packaging signals direct incorporation of viral genomes into virions 29

Core proteins may accompany the viral genome inside the capsid 29

Formation of viral envelopes by budding is driven by interactions between viral proteins 29

DISASSEMBLY OF VIRIONS: THE DELIVERY OF VIRAL GENOMES TO THE HOST CELL 30

Virions are primed to enter cells and release their genome 30

3. Virus Classification: The World of Viruses 32

VIRUS CLASSIFICATION 32

Many different viruses infecting a wide variety of organisms have been discovered 32

Virus classification is based on molecular architecture, genetic relatedness, and host organism 33

Viruses are grouped into species, genera, families and orders 33

Distinct naming conventions and classification schemes have developed in different domains of virology 34

MAJOR VIRUS GROUPS 34

Study of the major groups of viruses leads to understanding of shared characteristics and replication pathways 34

Viruses with single-stranded DNA genomes are small and have few genes 35

Viruses with double-stranded DNA genomes include the largest known viruses 35

Most plant viruses and many viruses of vertebrates have positive-strand RNA genomes 38

Viruses with negative-strand RNA genomes have helical nucleocapsids; some have fragmented genomes 39

Viruses with double-stranded RNA genomes have fragmented genomes and capsids with icosahedral symmetry 40

Viruses with a reverse transcription step in their replication cycle can have either RNA or DNA genomes 40

Satellite viruses and satellite nucleic acids require a helper virus to replicate 41

Viroids do not code for proteins, but replicate independently of other viruses 41

THE EVOLUTIONARY ORIGIN OF VIRUSES 42

The first steps in the development of life on Earth: the RNA world 42

Viroids and RNA viruses may have originated in the RNA world 43

The transition to the DNA-based world 44

Retroviruses could have originated during the transition to DNA-based cells 44

Small- and medium-sized DNA viruses could have arisen as independently replicating genetic elements in cells 44

Large DNA viruses could have evolved from cellular forms that became obligatory intracellular parasites 45

These arguments about the origin of viruses are only speculations 45

4. Virus Entry 47

How do virions get into cells? 47

Enveloped and non-enveloped viruses have distinct penetration strategies 48

Some viruses can pass directly from cell to cell 49

A variety of cell surface proteins can serve as specific virus receptors 49

Receptors interact with viral glycoproteins, surface protrusions, or “canyons” on the surface of the virion 50

Many viruses enter the cell via receptor-mediated endocytosis 50

Passage from endosomes to the cytosol is often triggered by low pH 52

Viral fusion proteins are cleaved by proteases prior to budding/ final assembly or during entry to reveal a fusion peptide 52

Fusion proteins undergo major conformational changes that lead to membrane fusion 53

Class I and class II fusion proteins have different structures and different fusion mechanisms 53

Non-enveloped viruses penetrate by membrane lysis or pore formation 54

Virions and capsids are transported within the cell in vesicles or on microtubules 55

Import of viral genomes into the nucleus 55

Summary of the many ways in which viral genomes are uncoated and released 56

SECTION II: VIRUSES OF BACTERIA AND ARCHAEA

5. Single-Stranded RNA Bacteriophages 60

A glorious past: the discovery of single-stranded RNA phages stimulated research into messenger RNA function and RNA replication 60

Single-stranded RNA phages are among the simplest known organisms 61

Two genera of single-stranded RNA phages have subtle differences 61

RNA phages have a simple capsid with icosahedral symmetry that contains a single copy of the maturation protein 62

RNA phages bind to the F-pilus and use it to insert their RNA into the cell 62

Phage RNA is translated and replicated in a regulated fashion 62

RNA secondary structure controls the translation of lysis and replicase genes 64

Ribosomes translating the coat gene disrupt secondary structure, allowing replicase translation 64

Ribosomes terminating coat translation can reinitiate at the lysis gene start site 65

Replication versus translation: competition for the same RNA template 66

Genome replication requires four host cell proteins plus the replicase 66

A host ribosomal protein directs polymerase to the coat start site 67

Polymerase skips the first A residue but adds a terminal A to the minus-strand copy 67

Synthesis of plus strands is less complex and more efficient than that of minus strands 67

The start site for synthesis of maturation protein is normally inaccessible to ribosomes 68

Synthesis of maturation protein is controlled by delayed RNA folding 68

Virions are formed by assembly of coat protein dimers around genome RNA 69

Single gene lysis and release of mature phage virions 70

Genomics and diversity of single-stranded RNA bacteriophages 70

Single gene lysis protein (Sgl) diversity 70

Progress in genomics reveals that single-stranded RNA phages are more diverse than previously reported 71

6. Microviruses 74

φX174: a tiny virus with a big impact 74

Overlapping reading frames allow efficient use of a small genome 75

φX174 binds to glucose residues in cell surface lipopolysaccharides 76

φX174 DNA delivery requires extensive structural rearrangements at the cell-interacting vertex 76

DNA transport requires the H-tube, an ephemeral virus-encoded DNA transporting conduit 77

Stage I DNA replication generates double-stranded replicative form DNA 78

Gene expression is controlled by the strength of promoters and transcriptional terminators 78

Replicative form DNAs are amplified via a rolling circle mechanism 78

Summary of viral DNA replication mechanisms 79

Procapsids are assembled using scaffolding proteins 79

Scaffolding proteins have a flexible structure 80

Single-stranded genomes are packaged into procapsids as they are synthesized 81

The evolution of a capsid assembly system using two scaffolding proteins 81

Role of the J protein in DNA packaging 81

Cell lysis caused by E protein leads to release of phage 82

Scaffolding proteins may increase the rate of assembly and help small phages compete against larger phages 82

7. Bacteriophage T7 84

T7: a model phage for DNA replication, transcription, and RNA processing 84

T7 genes are organized into three groups based on transcription and gene function 85

The T7 virion contains capsid, core, tail, and fiber proteins 86

Entry of T7 DNA into the cytoplasm is powered by transcription 86

One of the products of early transcription is a new T7 RNA polymerase 86

Other Class I genes inhibit host cell functions 87

T7 RNA polymerase transcribes class II and III genes 88

T7 RNAs are cleaved by the host enzyme ribonuclease III to smaller, stable mRNAs 88

Class II genes code for enzymes involved in T7 DNA replication 88

DNA replication starts at a unique internal origin and is primed by T7 RNA polymerase 89

Large DNA concatemers are formed during replication 90

Class III genes direct progeny virion assembly 90

Concatemer processing and DNA packaging require transcription by T7 RNA polymerase 90

Special features of the T7 family of phages 91

T7 and biotechnology 91

Phage display 92

8. Bacteriophage T4 94

Bacteriophage T4: a model virus that has played a pivotal role in discoveries in molecular biology 95

T4 virions bind to cells via tail fibers 96

The tail sheath contracts and phage DNA penetrates the cell wall via a lysozyme at the tip of the tail tube 96

T4 early genes are transcribed by host cell RNA polymerase 99

T4 early gene products modify host RNA polymerase to express T4 middle genes 100

Three T4 middle gene products activate late transcription 100

T4 gene products modulate messenger RNA stability 100

Three T4 genes contain self-splicing group I introns 101

T4 gene products repress translation of their own or other T4 mRNAs by binding near translation initiation sites 101

Secondary structures in T4 messenger RNAs control translation of specific genes 101

T4 DNA replication begins with RNA primers and is carried out by ten phage-coded proteins 103

DNA replication switches to a recombination mode using D-loop primers 104

T4 assembles a prohead at cell membrane with aid of chaperone proteins 105

Holin-antiholin interactions control cell lysis 106

T4 virus-like particles for phage display 107

T4 within the gut microbiome 108

9. Bacteriophage Lambda 110

Roots . . . 110

Phage adsorption and DNA entry depend on cellular proteins involved in sugar transport 111

The λ lytic transcription program is controlled by termination and antitermination of RNA synthesis at specific sites on the genome 112

The CI repressor blocks expression of the lytic program by regulating three nearby promoters: PL, PR, and PRM 113

A genetic switch: cleavage of CI repressor in cells with damaged DNA leads to prophage induction 115

The Cro repressor suppresses CI synthesis and regulates early gene transcription 115

Making the decision: go lytic or lysogenize? 115

A quick review 116

Breaking and entering: the insertion of λ prophage DNA into the bacterial chromosome 116

Excision of λ DNA from the bacterial chromosome 116

Int synthesis is controlled by retroregulation 118

λ DNA replication is directed by O and P but carried out by host cell DNA replication proteins 119

Assembly of λ heads involves chaperones and scaffolding proteins 119

DNA is inserted into preformed proheads by an ATP-dependent mechanism 119

Host cell lysis is mediated by a holin and a spanin 120

10. Viruses of Archaea 123

Archaea, the third domain of life 123

Viruses of archaea have diverse and unusual morphologies 124

Virions of Acidianus bottle-shaped virus (ABV) are released from host cells without cell lysis 125

Acidianus two-tailed virus (ATV) has a virion with tails that spontaneously elongate 125

Infection with ATV at high temperatures results in lysogeny 125

Aeropyrum pernix bacilliform virus 1 (APBV-1) is a rod-shaped virus with helical symmetry and a small double-stranded DNA genome 125

Sulfolobous spindle-shaped viruses (SSV) contain positively supercoiled DNA, and integrate their DNA into tRNA genes of the host cell 128

Transcription of SSV1 DNA is temporally controlled 130

Members of the *Globuloviridae* family have a spherical enveloped virion that contains a helical DNA-protein complex 130

Virions of the *Guttaviridae* have a droplet shape 131

Aeropyrum coil-shaped virus (ACV) has a helical nucleocapsid and the largest known single-stranded DNA genome 132

Pyrobaculum filamentous virus 1 (PFV1) is a lytic virus with an enveloped virion that encloses a helical nucleocapsid 133

Members of the *Turriviridae* have virions that resemble those of bacterial virus families 134

Sulfolobus islandicus rod-shaped virus 2 (SIRV2) has a helical capsid; progeny virions escape from infected cells via a specialized pyramidal structure 134

The lipothrixviruses and ungulaviruses have long filamentous virions that are enclosed in lipid-containing envelopes and exit the cell via pyramidal protrusions 135

Comparative genomics of archaeal viruses 136

CONCLUSION 137

SECTION III: POSITIVE-STRAND RNA ANIMAL VIRUSES

11. Picornaviruses 140

Picornaviruses cause a variety of human and animal diseases including poliomyelitis and the common cold 140

Poliovirus: a model picornavirus for vaccine development and studies of replication 141

Picornavirus virions bind to cellular receptors via depressions or loop regions on their surface 142

Genome RNA may pass through pores formed in cell membranes by capsid proteins 142

Translation initiates on picornavirus RNAs by a novel internal ribosome entry mechanism 143

Essential features of picornavirus IRES elements 144

Interaction of picornavirus IRES elements with host cell proteins 145

Picornavirus proteins are made as a single precursor polypeptide that is autocatalytically cleaved by viral proteinases 145

Picornaviruses encode several proteinases that cleave the polypeptide and some host cell proteins 146

Replication of picornavirus RNAs is initiated in a multiprotein complex bound to proliferated cellular vesicles 147

RNA synthesis is primed by VPg covalently bound to uridine residues 148

Virion assembly involves cleavage of VP0 to VP2 plus VP4 148

Inhibition of host cell macromolecular functions 149

Picornavirus pathogenesis and disease 150

12. Flaviviruses 152

Flaviviruses cause several important human diseases 153

Yellow fever is a devastating human disease transmitted by mosquitoes 153

A live, attenuated yellow fever virus vaccine is available and widely used 154

Dengue virus, a tropical virus encroaching upon developed countries in the wake of global warming and travel 154

Zika, a prime example of an emerging infectious disease with unforeseen disease manifestations 155

Hepatitis C virus: a recently discovered member of the *Flaviviridae* 155

The flavivirus virion contains a lipid bilayer and envelope proteins arranged with icosahedral symmetry 155

Flavivirus E protein directs both binding to receptors and membrane fusion 156

Flaviviruses enter the cell by pH-dependent fusion 157

Flavivirus genome organization resembles that of picornaviruses 157

Flavivirus genome translation is non-canonical 157

The polypeptide is processed by both viral and cellular proteases 158

Nonstructural proteins organize protein processing, viral RNA replication, and capping 160

Flavivirus RNA synthesis is carried out on membranes in the cytoplasm 160
 The flavivirus subgenomic RNA promotes virus infection 161
 Virus assembly also takes place at intracellular membranes 161

13. Hepaciviruses 164

Hepatitis C is a blood-borne disease that is mainly spread by people who inject drugs 165
 The discovery of hepatitis C virus led to a Nobel Prize in 2021 165
 Hepatitis C virus has a narrow host range and replicates only in humans and chimpanzees 166
 The Japanese fulminant hepatitis (JFH1) strain of HCV was found to grow in human hepatoma cells without the need for adaptive mutations 166
 Hepatitis C virions are roughly spherical but are pleomorphic and of varying size 166
 Nonstructural proteins are required for cleavage of the viral polyprotein, inhibition of host immunity, and replication of the HCV genome 167
 Virus entry begins with stepwise attachment to a series of receptors 169
 Following receptor binding, virions enter the cell via clathrin-mediated endocytosis 170
 Genome RNA is translated into a polyprotein that is processed into individual viral proteins by four different host and viral proteases 171
 RNA structural elements organize replication factors and interact with host cell microRNA-122 to stimulate HCV replication 171
 A membranous web derived from the endoplasmic reticulum is the site of virus replication 171
 Virus assembly requires both nonstructural and structural proteins associated with lipid droplets in the membranous web 171
 Egress and release of HCV lipoprotein particles 172
 HCV exhibits a high rate of mutation in the face of host immune surveillance and resists both neutralizing antibodies and components of innate immunity 173
 Mouse models allowing HCV replication were developed for testing antiviral drugs 174
 Antiviral drug therapies were facilitated by rational drug design based on the 3D structures of viral proteins 174
 Genetic variation has posed a barrier to vaccine development 175
 Other mammalian hepaciviruses are being discovered 175

14. Togaviruses and Rubella Virus 178

Transmission of togaviruses: role of mosquito vectors 179
 Rubella virus causes mild disease in humans but can cause serious birth defects 179
 Alphaviruses cause a variety of human diseases that include fatal encephalitis 180
 Alphaviruses are models for the study of virus-host interactions 180

Alphavirus virions contain a nucleocapsid with icosahedral symmetry wrapped in an envelope of the same symmetry 180
 Rubella virus virions lack icosahedral symmetry 181
 Several different cellular proteins have been identified as receptors for alphaviruses and rubella virus 182
 Alphaviruses use low pH-induced fusion inside endosomes to deliver their genomes into the cytoplasm 182
 Nonstructural proteins are made as a polyprotein that is cleaved by a viral proteinase 182
 Genome replication occurs in association with spherules on cellular membranes 184
 Signals on genome and antigenome RNAs coordinate RNA replication and transcription 184
 Trans-cleavage of NSP123 is required for positive-strand RNA synthesis 186
 Genome and subgenomic RNAs are capped by NSP1 and NSP2 186
 Structural proteins are produced from subgenomic RNA synthesized during the late phase of replication 187
 Subgenomic RNAs are preferentially and efficiently translated 187
 Virions are assembled at the plasma membrane and released by budding 188
 Alphaviruses inhibit host cell antiviral responses by diverse mechanisms 189
 Alphaviruses are used to generate self-amplifying RNA vaccines 190

15. Coronaviruses 192

Coronaviruses are primarily respiratory pathogens in humans 193
 Coronaviruses cause veterinary disease and have great economic consequences for pets and the food and farming industries 193
 A newly emerged coronavirus caused a limited but worldwide epidemic of severe acute respiratory syndrome (SARS) starting in 2002–2003 194
 A coronavirus transmitted by dromedaries subsequently emerged in the Arabian Peninsula and also caused a limited but worldwide epidemic 194
 SARS-CoV-2 emerged in late 2019 to cause the most widespread and severe pandemic in the 100 years since the 1918 “Spanish flu” 194
 Coronaviruses fall into four genera based on genome sequences 195
 Coronaviruses have large, single-stranded, positive-sense RNA genomes 195
 Coronaviruses have enveloped virions containing helical nucleocapsids 196
 Coronavirus virions contain multiple envelope proteins 196
 Coronavirus spike proteins bind to a variety of cellular receptors 198
 The virus envelope fuses with the plasma membrane or an endosomal membrane 198

The replicase gene is translated from genome RNA into a polyprotein that is processed by viral proteases 198

RNA polymerase, RNA helicase, and RNA-modifying enzymes are encoded by the replicase gene 199

Replication complexes are associated with cytoplasmic membranes 200

Genome replication proceeds via a full-length, negative-strand intermediate 200

Transcription produces a nested set of subgenomic mRNAs 200

Subgenomic mRNAs are transcribed from subgenomic negative-sense RNA templates made by discontinuous transcription 202

The discontinuous transcription model can explain recombination between viral genomes 203

Assembly of virions takes place at intracellular membrane structures 203

Coronaviruses can evolve rapidly because of high mutation rates and RNA–RNA recombination 203

Epidemic human coronaviruses could have arisen by mutation or recombination from bat coronaviruses 203

An ancestor of SARS-CoV-2 may have circulated in horseshoe bats but the pathway of its eventual transmission to humans remains a mystery 204

Numerous SARS-CoV-2 variants emerged during the pandemic 205

Effective vaccines against COVID-19 were rapidly produced and distributed 205

Repurposed drugs inhibit virus replication and reduce disease symptoms when administered early 206

SECTION IV: NEGATIVE-STRAND AND DOUBLE-STRANDED RNA ANIMAL VIRUSES

16. Paramyxoviruses and Pneumoviruses 210

The mononegaviruses: a group of related negative-strand RNA viruses 211

Measles is a serious childhood disease caused by a paramyxovirus 211

Canine distemper virus is a veterinary disease that emerged from Peru in 1761 211

Mumps is another childhood illness that has been controlled by vaccination 212

Parainfluenza viral infections are common in the young and elderly 212

Nipah and Hendra viruses are zoonotic, highly pathogenic, and originate from bats 212

Respiratory syncytial virus and metapneumovirus, members of the *Pneumoviridae* family, cause lower tract respiratory disease 212

Other paramyxoviruses have been extensively studied in the laboratory 213

Gene order is conserved among different paramyxoviruses and pneumoviruses 213

Paramyxoviruses and pneumoviruses are pleiomorphic 214

Genome RNA is contained within helical nucleocapsids 214

Viral envelope proteins are responsible for receptor binding and fusion with cellular membranes 214

Paramyxoviruses attach to the host cell using either sialic acid or specific cell surface proteins 216

Respiratory syncytial virus G protein binds to cellular chemokine receptor 217

The fusion precursor protein is activated by proteolytic cleavage to generate a primed pre-fusion protein 217

Paramyxoviruses and pneumoviruses access the cytoplasm by membrane fusion at the plasma membrane or less frequently with endosomes 217

Viral messenger RNAs are synthesized by an RNA polymerase packaged in the virion 218

Viral RNA polymerase initiates transcription exclusively at the 3' end of the viral genome 218

The promoter for plus-strand RNA synthesis consists of two sequence elements separated by one turn of the ribonucleoprotein helix 220

mRNAs are synthesized sequentially from the 3' to the 5' end of the genome RNA 220

The P/C/V gene codes for several proteins by using alternative translational starts and by mRNA “editing” 221

Functions of P, C, and V proteins 222

N protein levels control the switch from transcription to genome replication 223

Virions are assembled at the plasma membrane 223

17. Rhabdoviruses 226

Rabies is a fatal human encephalitis caused by a rhabdovirus that is transmitted by animal bites 227

Vesicular stomatitis virus is a representative rhabdovirus and one of the best-studied RNA viruses 228

Rhabdoviruses are bullet- or rod-shaped and have a helical nucleocapsid 228

Genome organization and the replication cycle of rhabdoviruses 229

Virus attachment and entry occur via pH-dependent endocytosis 229

The matrix protein inhibits the interferon antiviral response of the host cell 229

Viral genes are transcribed from intact ribonucleoprotein complexes into five mRNAs by a “start-stop” mechanism 230

Rhabdovirus virions are assembled at the plasma membrane 230

Nobel Prize-winning discoveries utilized the VSV G protein as a tool to study protein trafficking 231

Reverse genetics of rhabdoviruses allows the generation of recombinant viruses for basic research and a variety of biomedical applications 232

VSV G pseudotyped lentivirus expression and gene therapy vectors 232

Non-replicating VSV pseudotypes lacking G protein (Δ G-VSV) and replication-competent Δ G-VSV hybrid viruses 233

VSV pseudotypes lacking G protein (Δ G-VSV) and replication-competent Δ G-VSV hybrid viruses 233

Vesicular stomatitis virus-based recombinant vaccines 234

18. Filoviruses 237

Marburg and Ebola viruses: sporadically emerging viruses that cause severe, often fatal disease 238

Most filovirus outbreaks have occurred in equatorial Africa 238

Bats or other small mammals may be the animal reservoir from which filoviruses circulate to primates and humans 240

Filoviruses are related to paramyxoviruses and rhabdoviruses 240

Filoviruses cause hemorrhagic fever 241

Filovirus genomes contain seven genes in a conserved order 241

Filovirus transcription, replication, and assembly 242

Cloned cDNA copies of viral mRNAs and viral genome RNA are used to study filoviruses 243

Multiplasmid transfection systems allow recovery of infectious filoviruses 243

Filovirus glycoprotein mediates both receptor binding and entry by fusion 244

Ebola virus uses RNA editing to make three glycoproteins from the same gene 245

Do the secreted glycoproteins play a role in virus pathogenesis? 246

Minor nucleocapsid protein VP30 activates viral mRNA synthesis in Ebola virus 247

Matrix protein VP40 directs budding and formation of filamentous particles 247

Spread of filovirus infections among humans is limited to close contacts 248

Pathogenesis of filovirus infections 248

Clinical features of infection 248

19. Bunyaviruses 251

Most bunyaviruses are transmitted by arthropod vectors, including mosquitoes and ticks 252

Some bunyaviruses cause severe hemorrhagic fever, respiratory disease, or encephalitis 253

Bunyaviruses encapsidate a segmented RNA genome in a simple enveloped particle 253

Bunyavirus protein-coding strategies: negative-strand and ambisense RNAs 254

L RNA codes for viral RNA polymerase 254

M RNA codes for virion envelope glycoproteins 254

S RNA codes for nucleocapsid protein and a nonstructural protein 256

After attachment via virion glycoproteins, bunyaviruses enter the cell by endocytosis 256

Bunyavirus mRNA synthesis is primed by the capped 5' ends of cellular mRNAs 256

Coupled translation and transcription may prevent premature termination of mRNAs 257

Genome replication begins once sufficient N protein is made 258

Virus assembly takes place at Golgi membranes 258

Evolutionary potential of bunyaviruses via genome reassortment 258

20. Influenza Viruses 262

Influenza viruses cause serious acute disease in humans, and occasional pandemics 263

Influenza virus infections of the respiratory tract can lead to secondary bacterial infections 263

Orthomyxoviruses are negative-strand RNA viruses with segmented genomes 263

Eight influenza virus genome segments code for at least 11 different viral proteins 264

Hemagglutinin protein binds to cell receptors and mediates fusion of the envelope with the endosomal membrane 264

M2 is an ion channel that facilitates release of nucleocapsids from the virion 265

Nucleocapsids enter the nucleus, where mRNA synthesis and RNA replication occur 267

Capped 5' ends of cellular pre-messenger RNAs are used as primers for synthesis of viral mRNAs 268

Viral mRNAs terminate in poly(A) tails generated by "stuttering" transcription 269

Two influenza A mRNAs undergo alternative splicing in the nucleus 270

Genome replication begins when newly synthesized NP protein enters the nucleus 270

Nucleocapsids are exported from the nucleus in a complex with matrix protein and NS2 271

The NS1 protein interferes with polyadenylation of cellular mRNAs 271

The NS1 protein also suppresses a variety of host cell antiviral response pathways 271

PB1-F2 and PA-X proteins may contribute to suppression of the host immune response 271

Viral envelope proteins assemble in the plasma membrane and direct budding of virions 272

Neuraminidase cleaves sialic acid, the cellular receptor that binds to HA 272

Influenza virus strains vary in both transmissibility and pathogenicity 273

Genetic variability generates new virus strains that can cause pandemics 273

The 1918 pandemic influenza A virus was probably not a reassortant virus 273

Genome sequences from some previous influenza A virus strains confirm the antigenic shift hypothesis 274

Highly pathogenic avian influenza A H5N1 strains in poultry farms are a potential threat but are poorly transmitted among humans 274

A new pandemic strain of influenza A virus arose by genetic shift and spread worldwide in 2009 275

21. Reoviruses 278

- Reoviruses were the first double-stranded RNA viruses discovered 279
- Some reoviruses are important pathogens 279
- Members of the order *Reovirales* have segmented genomes 280
- Reovirus virions contain concentric layers of capsid proteins 282
- The fiber binds to one or two cellular receptors 282
- During entry, the outer capsid is stripped from virions and the core is released into the cytoplasm 283
- Enzymes in the viral core synthesize and cap messenger RNAs 283
- Translation of reovirus mRNAs is regulated 285
- Interferon and PKR: effects on viral and cellular protein synthesis 285
- Synthesis of progeny double-stranded genomes occurs within subviral particles 285
- Reoviruses induce and modulate apoptosis and interferon expression in infected cells 287
- Reovirus protein $\mu 1$ present in infecting virions induces apoptosis 287
- Reovirus pathogenesis in the mouse central nervous system depends on virus strain 287
- Reovirus infections in mice cause damage in heart and liver 288
- Reovirus infection can lead to autoimmune responses in mice 288

SECTION V: SMALL DNA ANIMAL VIRUSES

22. Parvoviruses 292

- Parvoviruses have very small virions and a linear, single-stranded DNA genome 292
- Parvoviruses replicate in cells that are going through the cell cycle 293
- Discovery of mammalian parvoviruses 293
- Parvoviruses have one of the simplest-known virion structures 294
- Parvoviruses have very few genes 294
- Single-stranded parvovirus DNAs have unusual terminal structures 295
- Uncoating of parvovirus virions takes place in the nucleus and is cell-specific 295
- DNA replication begins by extension of the 3' end of the terminal hairpin 296
- The DNA "end replication" problem 297
- Steps in DNA replication 297
- Nonstructural proteins are multifunctional 298
- Adenovirus functions that help replication of adeno-associated virus 298
- In the absence of helper virus, adeno-associated virus DNA can integrate into the cell genome 299
- Parvovirus pathogenesis: the example of B19 virus 299
- Vectors derived from adeno-associated viruses are highly efficient tools for *in vivo* gene delivery 300

23. Polyomaviruses 302

- Mouse polyomavirus was discovered as a tumor-producing infectious agent 303
- Simian virus 40 was found as a contaminant of Salk poliovirus vaccine 303
- Human polyomaviruses are widespread but cause disease only rarely 303
- Polyomaviruses are models for studying DNA virus replication and tumorigenesis 303
- Polyomavirus capsids are constructed from pentamers of the major capsid protein 304
- The circular DNA genome is packaged with cellular histones 304
- Circular DNA becomes supercoiled upon removal of histones 305
- Supercoiled DNA can be separated from relaxed or linear DNA molecules 305
- Polyomavirus genes are organized in two divergent transcription units 306
- Virions enter cells in caveolae and are transported to the nucleus 307
- The viral minichromosome is transcribed by cellular RNA polymerase II 308
- Four early mRNAs are made by differential splicing of a common transcript 308
- T antigens share common N-terminal sequences but have different C-terminal sequences 309
- T antigens bring resting cells into the DNA synthesis (S) phase of the cell cycle 309
- Small T antigen inhibits protein phosphatase 2A and induces cell cycling 309
- Middle T antigen stimulates protein tyrosine kinases that signal cell proliferation and division 310
- Large T antigen activates or suppresses transcription of cellular genes by binding to a number of important cellular regulatory proteins 311
- Large T antigen hexamers bind to the origin of DNA replication and locally unwind the two DNA strands 312
- Large T antigen assembles the cellular DNA synthesis machinery to initiate viral DNA replication 312
- High levels of late transcripts are made after DNA replication begins 314
- Three late mRNAs are made by alternative splicing 315
- How do polyomaviruses transform cells *in vitro* and cause tumors *in vivo*? 315
- Only nonpermissive cells can be transformed 316
- Transformed cells integrate viral DNA into the cell chromosome 316

24. Papillomaviruses 318

- Papillomaviruses cause warts and other skin and mucosal lesions 318
- Oncogenic human papillomaviruses are a major cause of genital tract cancers 319
- Papillomaviruses are not easily grown in cell culture 319
- Papillomavirus genomes are circular, double-stranded DNA 320

The infectious cycle follows the differentiation of epithelial cells 320

Viral mRNAs are made from two promoters and two polyadenylation signals 322

Viral E1 and E2 proteins bind to the replication origin and direct initiation of DNA replication 322

Viral E7 protein interacts with cell-cycle regulatory proteins, particularly the retinoblastoma protein pRB 323

The retinoblastoma protein is a major regulator of the cell cycle 323

Viral E6 protein controls the level of cellular p53 protein 324

p53 induces cell-cycle arrest or apoptosis (programmed cell death) 324

E6 proteins interact with many other cellular regulatory proteins 325

Synergism between E6 and E7 and the predisposition to cancer 325

Cells transformed by papillomaviruses express E6 and E7 gene products from integrated viral DNA 325

Future prospects for diagnosis and treatment of diseases caused by papillomaviruses 326

SECTION VI: LARGER DNA ANIMAL VIRUSES

25. Adenoviruses 330

Adenoviruses cause respiratory and enteric infections in humans 330

Adenoviruses can be oncogenic, but do not cause cancer in humans 331

Adenoviruses are versatile vectors for vaccination, gene therapy, and cancer treatment 331

Virions have icosahedral symmetry and are studded with knobbed fibers 331

Fibers make contact with cellular receptor proteins to initiate infection 332

Expression of adenovirus genes is controlled at the level of transcription 333

E1A proteins are the kingpins of the adenovirus growth cycle 334

E1A proteins bind to the retinoblastoma protein and activate E2F, a cellular transcription factor 334

E1A proteins also activate other cellular transcription factors 335

E1A proteins indirectly induce apoptosis by activation of cellular p53 protein 335

E1B proteins suppress E1A-induced apoptosis and target key proteins for degradation, allowing virus replication to proceed 336

The pre-terminal protein primes DNA synthesis carried out by viral DNA polymerase 336

Single-stranded DNA is circularized via the inverted terminal repeat 337

The major late promoter is activated after DNA replication begins 338

Five different poly(A) sites and alternative splicing generate multiple late mRNAs 338

The tripartite leader ensures efficient transport of late mRNAs to the cytoplasm 339

The tripartite leader directs efficient translation of late adenovirus proteins 339

Adenovirus-induced cell killing 339

Cell transformation and oncogenesis by human adenoviruses 339

Adenoviruses as mammalian cell expression vectors 340

26. Herpesviruses 344

Herpesviruses are important human and animal pathogens 345

Most herpesviruses can establish latent infections 345

HERPES SIMPLEX VIRUS 346

Herpes simplex virus genomes contain both unique and repeated sequence elements 346

The icosahedral capsid is enclosed in an envelope along with tegument proteins 346

Entry by fusion is mediated by envelope glycoproteins and may occur at the plasma membrane or in endosomes 348

Groups of viral genes are sequentially expressed during the replication cycle 349

Tegument proteins interact with cellular machinery to activate viral gene expression and degrade cellular messenger RNAs 349

Immediate early (α) genes regulate expression of other herpesvirus genes 350

β gene products facilitate viral DNA replication 350

DNA replication initially proceeds in a bidirectional fashion from a replication origin 351

Rolling-circle replication subsequently produces multimeric concatemers of viral DNA 351

DNA replication leads to activation of γ_1 and γ_2 genes 351

Viral nucleocapsids are assembled on a scaffold in the nucleus 352

Envelopment of nucleocapsids begins at the nuclear membrane and is completed at Golgi membranes 352

EPSTEIN-BARR VIRUS 354

Epstein-Barr virus was first discovered in lymphomas of African children 354

Epstein-Barr virus infects mucosal epithelial cells and B lymphocytes 355

Epstein-Barr virus expresses a limited set of proteins in latently infected B lymphocytes 355

Epstein-Barr virus nuclear antigens direct limited replication of the viral genome and activate viral and cellular genes 356

Latent membrane proteins mimic receptors on B lymphocytes 357

Small, untranslated viral RNAs expressed during latent infections target host defense mechanisms 357

KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS 358

Kaposi's sarcoma is a cancer that is frequently present in AIDS patients 358

Kaposi's sarcoma-associated herpesvirus infections are widespread but usually asymptomatic 358

Kaposi's sarcoma-associated herpesvirus infects epithelial cells and B lymphocytes 358

Kaposi's sarcoma-associated herpesvirus expresses many oncogenes 359

V-cyclin complexes with cyclin-dependent kinases and activates protein phosphorylation 359

Viral G protein-coupled receptor induces expression of cellular proteins that promote cell proliferation 360

VARICELLA-ZOSTER VIRUS 360

Chickenpox and shingles are human diseases caused by varicella-zoster virus 360

Varicella-zoster virus infects sensory neurons and establishes latent infections 360

Reactivation can cause shingles, a painful rash limited to a region innervated by a single nerve 361

HUMAN CYTOMEGALOVIRUS 361

Human cytomegalovirus infections are usually asymptomatic but can cause serious birth defects 361

ANTIVIRAL DRUGS DIRECTED AGAINST HERPESVIRUSES 362

VACCINES DIRECTED AGAINST HERPESVIRUSES 363

Varicella-zoster virus vaccines 363

Herpes simplex virus vaccines 363

Human cytomegalovirus vaccines 364

27. Poxviruses 366

Smallpox was a debilitating and fatal worldwide disease 367
Variolation led to vaccination, which has eradicated smallpox worldwide 367

Outbreaks of mpox infection show the importance of continued poxvirus surveillance 368

Other poxviruses infect either vertebrates or insects 368

Poxviruses are important research tools 369

Two forms of vaccinia virions have different roles in spreading infection 369

Virus entry into cells can take place by several different pathways 370

Linear vaccinia virus genomes have covalently sealed hairpin ends and lack introns 370

Poxviruses replicate and synthesize proteins in cytoplasmic factories; the first wave of gene expression occurs within viral cores 372

Early gene products promote genome uncoating and DNA replication 373

Poxviruses produce large concatemeric DNA molecules that are resolved into monomers 373

The transition to post-replicative gene expression requires DNA replication 374

Formation of mature virions is a complex, poorly understood process 375

Extracellular virions are extruded through the plasma membrane by actin tails 376

Poxviruses make several proteins that target host defenses against invading pathogens 376

Poxviruses perform an intricate dance with the host cell throughout infection 377

SECTION VII: VIRUSES WITH A REVERSE TRANSCRIPTASE

28. Retroviruses 382

Retroviruses have a unique replication cycle based on reverse transcription and integration of their genomes 383

Viral proteins derived from the gag, pol, and env genes are incorporated in virions 383

Retroviruses enter cells by the fusion pathway 384

Viral RNA is converted into a double-stranded DNA copy by reverse transcription 384

A copy of proviral DNA is integrated into the cellular genome at a random site 387

Sequence elements in the long terminal repeat direct transcription and polyadenylation by host cell enzymes 388

Differential splicing generates multiple mRNAs 389

The Gag/Pol polyprotein is made by suppression of termination and use of alternative reading frames 389

Virions mature into infectious particles after budding from the plasma membrane 389

Acute transforming retroviruses express mutated forms of cellular growth signaling proteins 390

Retroviruses lacking oncogenes can transform cells by insertion of proviral DNA near a proto-oncogene 391

29. Human Immunodeficiency Virus 394

Human immunodeficiency virus type 1 and acquired immunodeficiency syndrome 395

HIV-1 was probably transmitted to humans from chimpanzees infected with simian immunodeficiency virus 395

HIV-1 infection leads to a progressive loss of cellular immunity and increased susceptibility to opportunistic infections 395

Antiviral drugs can control HIV-1 infection and prevent disease progression, but an effective vaccine has yet to be developed 396

HIV-1 is a complex retrovirus 397

HIV-1 targets cells of the immune system by recognizing CD4 antigen and chemokine receptors 398

Virus mutants arise rapidly because of errors generated during reverse transcription 398

Unlike other retroviruses, HIV-1 directs transport of proviral DNA into the cell nucleus 399

Latent infection complicates the elimination of HIV-1 399

The Tat protein increases HIV-1 transcription by stimulating elongation by RNA polymerase II 400

The Rev protein mediates cytoplasmic transport of viral mRNAs that code for HIV-1 structural proteins 401

Together, the Tat and Rev proteins strongly upregulate viral protein expression 401

The Vif protein increases virion infectivity by counteracting a cellular deoxycytidine deaminase 402

The Vpr protein enhances HIV-1 replication at multiple levels 402

The Vpu protein enhances release of progeny virions from infected cells 402

The Nef protein is an important mediator of pathogenesis 403

30. Hepadnaviruses 406

At least seven distinct viruses cause human hepatitis 407

The discovery of hepatitis B virus 407

Hepatitis B virus has a circular double-stranded DNA genome and uses a reverse transcriptase to replicate 407

Dane particles are infectious virions; abundant non-infectious particles lack nucleocapsids 408

Distinct sequence variants of Hepatitis B virus occur in specific regions of the world 408

The viral genome is a circular, partly single-stranded DNA with overlapping reading frames 408

Nucleocapsids enter the cytoplasm via fusion and are transported to the nucleus 409

The viral genome becomes a fully covalently-closed DNA within the nucleus 410

Transcription of viral DNA gives rise to several mRNAs and a pregenome RNA 410

The roles of hepatitis B virus proteins 410

The pregenome RNA is packaged by interaction with polymerase and core proteins 412

Genome replication occurs via reverse transcription of pregenome RNA 412

Virions are formed by budding in the endoplasmic reticulum 414

Hepatitis B virus can cause chronic or acute hepatitis, cirrhosis, and liver cancer 416

Hepatitis B virus is transmitted by blood transfusions, contaminated needles, and unprotected sex 416

A recombinant vaccine is available 416

Antiviral drug treatment has real success 416

SECTION VIII: VIROIDS AND PRIONS

31. Viroids and Hepatitis Delta Virus 420

Viroids are small, circular RNAs that do not encode proteins 421

The two families of viroids have distinct properties 421

Viroids replicate via linear multimeric RNA intermediates 422

Three enzymatic activities are needed for viroid replication 422

How do viroids cause disease? 424

Viroid RNAs interact with host cell components and induce biochemical changes 424

RNA interference could determine viroid pathogenicity and cross-protection 424

Circular plant satellite RNAs resemble viroids but are encapsidated 425

Hepatitis delta virus is a human viroid-like satellite virus 426

Hepatitis delta virus may use two different cellular RNA polymerases to replicate 426

RNA editing generates two forms of hepatitis delta antigen 427

Delta-like agents: viruses similar to hepatitis delta virus found in non-human hosts 428

Conclusion: viroids and the hepatitis delta virus may be a link to the ancient RNA world 428

32. Prions 431

Prions are proteins that cause fatal brain diseases 431

Prion diseases were first detected in domestic ruminants 432

Bovine spongiform encephalopathy (“mad cow disease”) developed in Britain and apparently spread to humans 432

Human prion diseases can be either inherited or transmitted 432

The infectious agent of prion diseases contains protein but no detectable nucleic acid 433

PrPSc is encoded by a host cell gene 434

Distinct 3-dimensional structures of PrPC and PrPSc 434

The prion hypothesis: formation of infectious and pathogenic prions from normal PrPC 435

Is the prion hypothesis correct? 436

Pathology and diagnosis of prion diseases 438

Proteins of yeast and other fungi can form self-propagating states resembling prions 439

Genetics of prion diseases in mammals: mutations in the prion gene can increase occurrence of disease 439

Barriers to prion transmission between different species 439

Strain variation and crossing of the species barrier 440

The nature of the prion infectious agent 440

Can prion diseases be prevented or cured? 441

SECTION IX: VIRUSES OF PLANTS, ALGAE, AND INVERTEBRATES

33. Cucumber Mosaic Virus 444

Mosaic disease in cucumber plants led to the discovery of cucumber mosaic virus 445

Cucumber mosaic virus has a positive-strand RNA genome enclosed in a compact capsid with icosahedral symmetry 445

The genome of cucumber mosaic virus consists of three distinct RNA molecules 446

The three genome RNAs and a subgenomic RNA are encapsidated in separate but otherwise identical particles 447

The 3'-terminal regions of cucumber mosaic virus genome segments can fold to form a transfer RNA-like structure 447

Cucumber mosaic virus is transmitted in nature by aphids 448

The genome of cucumber mosaic virus encodes five multifunctional proteins 448

Replication of viral RNA is associated with intracellular membranes, and requires coordinated interaction of viral RNAs, proteins, and host proteins 449

Brome mosaic virus RNA replication has been analyzed in yeast cells 449

Brome mosaic virus RNA synthesis takes place on cytoplasmic membranes 450

Packaging of viral genomes 451

Cucumber mosaic virus requires protein 3a and coat protein for cell-to-cell movement and for long-distance spread within infected plants 451

Tobacco mosaic virus movement protein can direct movement of cucumber mosaic virus in infected plants 452

Mutation, recombination, reassortment, and genetic bottlenecks are involved in the evolution of cucumber mosaic virus 452

Host responses to cucumovirus infections reflect both a battle and adaptation between viruses and hosts 453

Plants respond to virus infection by RNA silencing, and cucumber mosaic virus protein 2b suppresses silencing 454

Cucumber mosaic virus supports replication of defective and satellite RNAs 454

Satellite RNAs can either attenuate or increase severity of symptoms in infected plants 455

34. Viruses of Algae and Mimivirus, a Giant Virus 457

Aquatic environments harbor large viruses 458

Phycodnaviruses are diverse and probably ancient 458

Phycodnavirology: a field in its infancy 459

Conserved structure, diverse composition 459

CHLOROVIRUSES 459

Chloroviruses replicate in *Chlorella* isolated from symbiotic hosts 459

The linear genomes of chloroviruses contain hundreds of genes, and each virus species encodes some unique proteins 460

Chlorovirus capsids are constructed from many capsomers and have a unique spike 461

Virus entry begins by binding to and degradation of the host cell wall 462

Transcription of viral genes is temporally controlled and probably occurs in the cell nucleus 462

Progeny virions are assembled in the cytoplasm 463

Chlorovirus proteins are small and efficient 464

A virus family with a penchant for sugar metabolism: hyaluronan and chitin 464

COCCOLITHOVIRUSES 464

Viruses that control the weather 464

Many genes looking for a function 465

Expression of coccolithovirus genes is temporally regulated 466

Cheshire Cat dynamics: sex to avoid virus infection 467

Survival of the fattest: the giant coccolithovirus genome encodes sphingolipid biosynthesis 467

PRASINOVIRUSES 468

Small host, big virus 468

Viral genomes contain multiple genes for capsid proteins 468

It works both ways 469

Not much room for maneuver 469

PHAEOVIRUSES 469

Seaweed viruses 469

Phaeoviruses have a temperate life cycle and integrate their genomes into the host DNA 470

Wide range in genome size and highly divergent 470

PRYMNESIOVIRUSES

AND RAPHDIVIRUSES 470

The lesser-known Phycodnaviridae 470

MIMIVIRUSES 471

A discovery marking the dawn of the megavirus! 471

Mimivirus is unquestionably a virus 472

Why such a large genome? 472

Mimivirus has a unique mechanism for releasing its core 473

Virus replication occurs exclusively in the cytoplasm 474

Genes coding for translation factors and DNA repair enzymes 474

Ancestors of mimivirus may have transferred genes from bacteria to eukaryotes 476

CONCLUSION 476

35. Baculoviruses 478

Insect viruses were first discovered as pathogens of silkworms 479

Baculoviruses are used for pest control and to express eukaryotic proteins 479

Baculovirus virions contain an elongated nucleocapsid 480

Baculoviruses produce two kinds of particles: "budded" and "occlusion-derived" virions 480

Baculoviruses have large, circular DNA genomes and encode many proteins 481

Insects are infected by ingesting occlusion bodies; infection spreads within the insect via budded virions 481

Viral proteins are expressed in a timed cascade regulated at the transcription level 482

Immediate early gene products control expression of early genes 483

Early gene products regulate DNA replication, late transcription, and apoptosis 483

Late genes are transcribed by a novel virus-coded RNA polymerase 484

Baculoviruses are widely used to express foreign proteins 485

36. Viruses of Invertebrates 489

Invertebrate viruses outnumber the viruses found in both vertebrates and plants 489

Hosts infected by invertebrate viruses include a wide variety of organisms 489

VIRUSES WITH DOUBLE-STRANDED DNA GENOMES 490

Ascoviridae are spread from parasitic wasps to lepidopteran larval hosts 490

Entomopoxvirinae infect the larvae of their hosts and are embedded in occlusion bodies called spheroids 492

Iridoviridae are large iridescent viruses that replicate starting in the nucleus and then in the cytoplasm 493

Baculoviridae have proven to be commercially valuable in the agriculture, forestry, and biotechnology sectors 493

Hytrosaviridae infect the salivary glands of the tsetse fly and common house fly 494

Malacoherpesviridae are an important threat to the mollusk seafood industry 495

Nimaviridae cause white spot disease in shrimp 495

Nudiviridae have saved coconut plantations of the South Pacific but ravaged the crab and lobster fishing industries 495

Polydnaviriformidae have a symbiotic relationship with parasitic wasps and caterpillars 497

VIRUSES WITH SINGLE-STRANDED DNA GENOMES 498

Densovirinae, members of the Parvoviridae family, infect many different invertebrates 498

Bidensovirus is a single-stranded DNA insect virus in the *Bidnaviridae* family 499

VIRUSES WITH DOUBLE-STRANDED RNA GENOMES 499

Birnaviridae are unusual in that they have a bisegmented RNA genome 499

Sedoreoviridae are reoviruses that infect invertebrates like leafhoppers, mosquitoes, and ticks, which can serve as vectors for transmission to plants and mammals 499

Spinareoviridae are spiked reoviruses that can infect mollusks, crustaceans, ticks, mosquitoes, and wasps 499

VIRUSES WITH POSITIVE-SENSE RNA GENOMES 500

Roniviridae infect shrimp and crabs and have an envelope containing two glycoproteins 500

Dicistroviridae have a discistronic genome and infect a wide range of insects and crustaceans 500

Iflaviridae are one of the causes of colony collapse disorder in honeybee hives 501

Polycipiviridae mainly cause infections in ants 501

Extra small virus causes white tail disease in shrimp and prawns 502

Nodaviridae have been used to study RNA replication, RNA interference, insect control, and vaccine development 502

VIRUSES WITH NEGATIVE-SENSE RNA GENOMES 503

Lispiviridae are negative-strand RNA viruses discovered by megasequencing of arthropods 503

Nyamiviridae are found exclusively in invertebrates and replicate in the nucleus 503

VIRUSES THAT USE A REVERSE TRANSCRIPTASE TO REPLICATE THEIR GENOMES 503

Three invertebrate virus families include mostly retrotransposons 503

SECTION X: HOST DEFENSES AGAINST VIRUS INFECTION

37. Innate Immune Responses Against Virus Infection 506

INNATE CELLULAR DEFENSE SYSTEMS AGAINST VIRAL INFECTIONS 507

DETECTION OF VIRUS INFECTION BY HOST CELLS 507

Host cell pattern recognition receptors and a variety of other molecular detection systems sense virus infection 507

A variety of toll-like receptors recognize distinctive molecules produced during virus infections 508

RIG-1-like receptors detect double-stranded RNAs 509

Cytosolic DNA sensors: the second messenger cGAMP and the cGAMP synthase pathway 510

Other sensors also recognize viral double-stranded DNA 510

C-type lectin receptors including DC-SIGN recognize carbohydrates on virus particles 510

RESPONSE OF THE CELL TO VIRUS INFECTION 511

Multimers of pathogen recognition receptors transmit signals that lead to activation of cytokine genes 511

Stimulator of interferon genes (STING) is activated by binding to cyclic GMP-AMP 512

Cellular recognition of virus infection leads to production of cytokines 513

Recognition of virus infection can trigger death of infected cells 514

Other antiviral signal transduction pathways involve
“inflammasomes” 514

INTERFERONS 515

Virus-infected cells secrete interferons, which protect nearby
cells against virus infection 515

Interferons α , β , γ , and λ are made by different cells, bind to
different receptors, and have distinct functions 516

Transcriptional activation occurs by binding of transcription
factors to interferon gene enhancers 516

Interferon signal transduction is carried out via the Jak–Stat
pathway 517

Antiviral activities induced by interferons 518

Interferons have diverse effects on the immune system 520

RNA INTERFERENCE 520

Small interfering RNAs are involved in combating virus
infections in plants and invertebrates 520

Micro RNAs are used to control gene expression
in vertebrates 521

VIRUS STRATEGIES TO COUNTER HOST DEFENSES 522

Viral proteins inhibit molecular adaptors and impair
transduction signals downstream of pattern recognition
receptors 523

Viruses target receptors or adaptors of antiviral signals
leading to degradation in the ubiquitin/proteasome
pathway 523

Viruses regulate posttranscriptional modifications involved
in host antiviral responses 523

Viruses have evolved strategies to hide their nucleic acids
from detection by host sensors 524

38. Adaptive Immune Responses to Virus Infection 527

Multicellular organisms have evolved immune defenses against
viruses and other pathogens 528

Innate immune responses are rapid and often nonspecific 528

Adaptive immune responses are slower but recognize specific
pathogens 528

Specialized cell types take part in the host immune
response 528

Natural killer cells recognize virus-infected cells and kill them
via apoptosis pathways 530

Recognition of viral antigens by the adaptive immune
system 530

Primary and secondary organs of the immune system harbor B
and T lymphocytes 530

The major histocompatibility system and distinction of self
versus nonself 531

Two pathways for antigen processing and presentation:
endogenous and exogenous 532

Dendritic cells initiate antigen-specific responses 532

B and T lymphocytes have specific surface receptors that
recognize antigens 532

T lymphocytes respond to peptides on the surface
of antigen-presenting cells 533

Helper T cells are generated upon interaction of CD4 T cells
with MHC-II-bound peptides 533

Cytotoxic T cells are generated upon interaction of CD8
T cells with MHC-I-bound peptides 534

B lymphocytes respond to antigens and are stimulated to
differentiate into plasma cells by interaction with Tfh
cells 535

Antibodies come in a variety of forms 536

The enormous diversity of antibody specificities 536

VIRUS STRATEGIES TO COUNTER HOST DEFENSES 537

Some viruses can escape host immune responses by becoming
latent 537

Interference with antigen processing and presentation allows
viruses to escape cell-mediated immunity 537

Viruses make proteins that mimic cytokines, cytokine
receptors, and chemokines and interfere with host
defenses 537

Viruses evade antibody responses by targeting complement
and Fc receptors 538

Viruses evade adaptive immune responses by changing their
antigenic structure 538

SECTION XI: MEDICAL APPLICATIONS OF VIROLOGY

39. Antiviral Vaccines 542

Epidemics of infectious diseases arose as human populations
grew, cities became crowded, and travel became more
rapid 543

Crude types of vaccination against viral and parasitic
diseases began to be developed as early as the 16th
century 544

Edward Jenner and Louis Pasteur introduced the first effective
antiviral vaccines 544

Embryonated chicken eggs and cell culture played
major roles in vaccine development in the twentieth
century 545

Production of vaccines against avian influenza strains has been
problematic 545

TYPES OF ANTIVIRAL VACCINES 546

Advantages and drawbacks of “classical” vaccine types 549

Newer categories of antiviral vaccines 549

HOW DO ANTIVIRAL VACCINES WORK? 550

The role of the immune system in fighting
viral infections 551

Adjuvants play an important role in vaccination
with inactivated or subunit vaccines 551

Vaccines that stimulate cell-mediated immunity are being
developed 552

NEW DEVELOPMENTS IN ANTIVIRAL VACCINES 552

New adjuvants are being developed 552

New delivery systems for viral antigens 554

- Vaccination with defined proteins 554
- Use of live viruses with defined attenuation characteristics 554
- Use of live vectors and chimeric viruses 554
- Vaccines that can break tolerance 555
- The changing vaccine paradigm 555

ADVERSE EVENTS AND ETHICAL ISSUES 556

- Vaccine-associated adverse events 556
- Ethical issues in the use of antiviral vaccines 557
- Experience gained during the COVID-19 pandemic 558

40. Antiviral Chemotherapy 562

DISCOVERY AND USES OF ANTIVIRAL DRUGS 562

- Despite challenges, antiviral drugs are a success story 562
- Antiviral drugs are useful for discoveries in basic research on viruses 563
- How antiviral drugs are obtained 563

TARGETS AND MECHANISMS OF ANTIVIRAL DRUGS 564

- Antiviral drugs are targeted to specific stages of virus infection 564
- Drugs preventing attachment and entry of virions 564
- Amantadine blocks an ion channel and inhibits uncoating of influenza 565
- Drugs blocking viral gene expression 566
- Baloxavir* blocks cap-snatching by influenza virus 567
- NS3/4 inhibitors and nirmatrelvir block cleavage of HCV and SARS-CoV-2 polyproteins 567

INHIBITORS OF GENOME REPLICATION 567

- Most nucleoside analog drugs target viral DNA or RNA polymerases 567
- Acyclovir is selectively phosphorylated by herpesvirus thymidine kinases 568
- Acyclovir is preferentially incorporated by herpesvirus DNA polymerases 569
- HIV reverse transcriptase preferentially incorporates azidothymidine into DNA, leading to chain termination 570
- Nucleoside analogs that are not obligate chain terminators 571
- Non-nucleoside inhibitors selectively target viral replication proteins 572

DRUGS THAT INTERFERE WITH VIRUS ASSEMBLY AND EGRESS 573

- HIV protease inhibitors were developed by rational methods 573
- Neuraminidase inhibitors block release and spread of influenza virus 574

ANTIVIRAL CHEMOTHERAPY: CURRENT SUCCESSES AND PROMISE FOR THE FUTURE 574

41. Oncolytic Viruses 578

- Oncolytic viral therapy defined 578
- Oncolytic viral activity was discovered through anecdotal reports in the literature 578
- Oncolytic viruses originate from a wide variety of virus families 579
- Oncolytic viruses act via several distinct mechanisms 580
- Mutations in Ras GTPases are found in many cancers and favor the replication of oncolytic viruses 580
- Replication of some oncolytic viruses can lead to cell death in cancer cells 581
- Targeting blood vessels in tumors using oncolytic viruses 582
- The immune system can control the fate of a cancer cell by eliminating it, living with it, or ignoring it 582
- Immunogenic cell death stimulates the recognition and processing of tumor antigens by antigen-presenting cells to produce long-lasting immunity 582
- Clinical efficacy and the landscape of oncolytic virus therapy 583
- Future prospects for oncolytic virus therapies 584

42. Virus-Mediated Gene Therapy 586

- Gene therapy defined 587
- Gene therapy strategies 587
- In vivo and ex vivo gene therapy 587
- Pioneers of retrovirus-mediated gene therapy 588
- Gene therapy using recombinant adeno-associated viruses 589
- Gene therapies for various cancers progressed to chimeric antigen receptor T-cell therapies 590
- Safety issues with gene therapy vectors 591
- How are gene therapy vectors designed and produced? 591
- Adeno-associated virus vectors can be directed to a variety of human tissues, and some can cross the blood-brain barrier 593
- Current FDA-approved human gene therapies 593
- Other treatments are on the horizon 594
- CRISPR/Cas9 gene editing is derived from a prokaryotic anti-phage defense mechanism 595
- CRISPR gene therapy relies upon viral vectors and a variety of gene delivery methods 596
- CRISPR/Cas therapies are beginning to be approved for treatment 596
- Concluding thoughts for the future of gene therapy 597

GLOSSARY 599

CREDITS AND PERMISSIONS 615

NAME INDEX 624

SUBJECT INDEX 626