THE VIRUSES

Series Editors:
HEINZ FRAENKEL-CONRAT
and ROBERT R. WAGNER

The Influenza Viruses

The Influenza Viruses

Edited by
ROBERT M. KRUG
Memorial Sloan-Kettering Cancer Center
New York, New York

PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

The Influenza viruses / edited by Robert M. Krug.
p. cm. – (The Viruses)
Includes bibliographies and index.
ISBN 0-306-43191-2
1. Influenza viruses. 2. Molecular biology. 3. Viral genetics. 4. Orthomyxoviridae – genetics. 5. Orthomyxoviridae – physiology. I. Krug, Robert M. II. Series.
[DNLM: QW 168.5.07 1435]
QR201.I6I535 1989
616.2'030194 – dc20
DNLM/DLC 89-8702
for Library of Congress CIP

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

Printed in the United States of America

Contributors

Ramesh K. Akkina, Department of Microbiology, College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado 80523

Firelli V. Alonso-Caplen, Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Thomas M. Chambers, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101

P. M. Colman, CSIRO Division of Biotechnology, Parkville 3052, Australia

Mary-Jane Gething, Department of Biochemistry and Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9038

Charles J. Hackett, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

Ilkka Julkunen, Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Michael G. Katze, Department of Microbiology, School of Medicine, University of Washington, Seattle, Washington 98195

Robert M. Krug, Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Robert A. Lamb, Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois 60208

Debi P. Nayak, Department of Microbiology and Immunology, Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, California 90024

Peter Palese, Department of Microbiology, Mount Sinai School of Medicine, New York, New York 10029

Michael G. Roth, Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9038

Joe Sambrook, Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9038

J. J. Skehel, Division of Virology, National Institute for Medical Research, London NW7 1AA, England

Frances I. Smith, Department of Microbiology, Mount Sinai School of Medicine, New York, New York 10029

W. Weis, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138

S. A. Wharton, Division of Virology, National Institute for Medical Research, London NW7 1AA, England

D. C. Wiley, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138

Jonathan W. Yewdell, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland 20852

Preface

Influenza virus is an important human pathogen, frequently causing widespread disease and a significant loss of life. Much has been learned about the structure of the virus, its genetic variation, its mode of gene expression and replication, and its interaction with the host immunologic system. This knowledge has the potential of leading to approaches for the control of influenza virus. In addition, research on influenza virus has led to important advances in eukaryotic molecular and cellular biology and in immunology.

A major focus of this book is the molecular biology of influenza virus. The first chapter, which serves as an introduction, describes the structure of each of the genomic RNA segments and their encoded proteins. The second chapter discusses the molecular mechanisms involved in the expression and replication of the viral genome. In addition to other subjects, this chapter deals with one of the most distinctive features of influenza virus, namely the unique mechanism whereby viral messenger RNA synthesis is initiated by primers cleaved from newly synthesized host-cell RNAs in the nucleus. Among the most significant accomplishments in influenza virus research has been the delineation of the three-dimensional structure of the two surface glycoproteins of the virus, the hemagglutinin and neuraminidase. This has provided a structural basis for mapping both the antigenic sites and the regions involved in the major biological functions of these two molecules. The current state of research on the hemagglutinin and neuraminidase is presented in the third and fourth chapters of this book. Chapter 5 describes the research on the biosynthesis, processing, and transport of these two viral glycoproteins. This research has been important not only for understanding the morphogenesis of influenza virus, but also for providing new information about the biosynthesis and transport of all cell-surface glycoproteins.

Defective-interfering virus particles were first discovered in influenza virus preparations, and the sixth chapter describes what is currently known about the generation and mechanism of action of influenza virus defective-interfering particles. The different evolutionary patterns of ge-

netic variation of influenza A, B, and C viruses in humans, and the roles of different influenza virus genes in pathogenicity, are dealt with in Chapter 7. Influenza virus induces thymus-derived lymphocytes (T lymphocytes), which play an important role in antiviral immunity in humans. As discussed in Chapter 8, studies using influenza A viruses have provided some of the most significant findings on the nature of the antigens recognized by T lymphocytes, on the specificity of T lymphocytes, and on the process by which antigens are presented on the cell surface for recognition by T lymphocytes.

This book was put together with two overlapping objectives in mind: providing a current review of the research on influenza virus for virologists, while highlighting for a wide audience of scientists the impact that influenza virus research has had on eukaryotic molecular and cellular biology and on immunology.

Robert M. Krug

Contents

Chapter 1

Genes and Proteins of the Influenza Viruses

Robert A. Lamb

I. Introduction 1
A. Structure of the Genome 2
II. RNA Segments 1, 2, and 3: The Three Polymerase-Associated Proteins Form a Complex to Transcribe RNA ... 6
A. Three P Proteins 7
B. PB1, PB2, and PA Gene Sequences 7
C. Functions of the PB1, PB2, and PA Proteins 8
D. P Proteins Form a Complex That Migrates to the Cell Nucleus 8
E. Influenza B Virus P Proteins and RNA Segments 10
F. Influenza C Viruses 10
III. RNA Segment 4: Synthesis, Structure, and Function of the Hemagglutinin 11
A. General Introduction to the Hemagglutinin 11
B. RNA Segment 4 Gene Structure 12
C. Three-Dimensional Structure of the Hemagglutinin 16
D. Synthesis of HA, Co-translational and Post-translational Modifications, Oligomerization, and the Exocytotic Pathway 18
E. Cleavage Activation 19
F. Viral Entry and Membrane Fusion 20
G. Influenza B Virus Hemagglutinin 20
H. Influenza C Virus Glycoprotein 20
IV. RNA Segment 5: The Nucleocapsid Protein Forms the Structural Monomer Unit of the Ribonucleoprotein Particles 22
A. Structure of RNPs and Involvement in RNA Synthesis .. 22
B. Properties of the Nucleocapsid Protein 23

C. Nuclear Transport and Karyophilic Sequences 24
D. RNA–Protein Interactions 25
E. NP Is a Major Target for Cytotoxic T Lymphocytes 26
F. Influenza B Virus NP Gene and Protein 27
G. Influenza C Virus NP Gene and Protein 27
V. RNA Segment 6: The Neuraminidase 28
A. Neuraminidase Function 28
B. Structure of Neuraminidase 28
C. General Deductions from the Gene Sequence of Influenza A Virus NA 29
D. Influenza A Virus Strain Similarities and Differences ... 30
E. N-Terminal Signal Anchor 31
F. Three-Dimensional Structure of NA 32
G. Influenza B Virus Neuraminidase 32
H. Influenza B Virus RNA Segment 6 33
I. NB Glycoprotein of Influenza B Virus 34
J. Effect of Mutations and Deletions in the Bicistronic mRNA for NB and NA 38
K. Influenza C Virus 39
VI. RNA Segment 7 of Influenza A Virus: Structure and Synthesis of the Membrane Protein (M_1) and an Integral Membrane Protein (M_2) from Unspliced and Spliced mRNAs 39
A. RNA Segment 7 and Its mRNAs 40
B. Membrane Protein (M_1) 42
C. Influenza Virus M_2 Protein Structure and Subcellular Localization 43
D. Expression of M_2 in Eukaryotic Cells from Cloned cDNA 47
E. Characterization of a Monoclonal Antibody to the M_2 Extracellular Domain 49
F. Initial Interaction of M_2 with Membranes 49
G. Is M_2 a Structural Component of Virions? 51
H. Function of M_2 in Infected Cells 52
I. Effect of Amantadine Hydrochloride on M_2 53
J. Evolution of the Nucleotide Sequence of RNA Segment 7 and the Amino Acid Sequences of M_1 and M_2 55
K. RNA Segment 7 of Influenza B Virus 56
L. Membrane Protein of Influenza C Virus 57
VII. Influenza A Virus RNA Segment 8: Unspliced and Spliced mRNAs Encode Nonstructural Proteins NS_1 and NS_2 58
A. RNA Segment 8 and Its mRNAs 58
B. NS_1 Protein 61
C. NS_2 Protein 64
D. RNA Segment 8 of Influenza B Virus: Unspliced and Spliced mRNAs Code for NS_1 and NS_2 65

E. Influenza C Virus RNA Segment 7: Unspliced and Spliced mRNAs Code for NS_1 and NS_2 66
References 67

Chapter 2

Expression and Replication of the Influenza Virus Genome

Robert M. Krug, Firelli V. Alonso-Caplen, Ilkka Julkunen, and Michael G. Katze

I. Introduction 89
II. Viral mRNA Synthesis 91
III. Template RNA Synthesis 98
IV. Virion RNA Synthesis 106
V. Regulation of Viral Gene Expression in Infected Cells 110
VI. Interferon-Induced Mx Protein, a Specific Inhibitor of Influenza Virus Replication 116
VII. Regulated Splicing of the Viral NS1 and M1 mRNAs 123
VIII. Mechanisms for the Selective and Efficient Translation of Influenza Virus mRNAs 133
References 142

Chapter 3

Structure, Function, and Antigenicity of the Hemagglutinin of Influenza Virus

S. A. Wharton, W. Weis, J. J. Skehel, and D. C. Wiley

I. Introduction 153
II. Structure of Hemagglutinin 153
III. Antigenic Variation 156
A. Antigenic Shift 156
B. Antigenic Drift 156
C. The Importance of Receptor Specificity in Antigenic Change 158
IV. Receptor Binding 159
A. Structure of the Receptor-Binding Site 160
B. The Binding Site of a Receptor-Binding Mutant 162
V. Fusion Properties of Hemagglutinin 164
A. Acid-Induced Conformational Change of Hemagglutinin 165
VI. Conclusions 169
References 169

Chapter 4

Neuraminidase: Enzyme and Antigen

P. M. Colman

I. Introduction 175
A. Biological Properties 176
B. Physical Characteristics 178
C. Gene Structure 179
II. Protein Structure 182
A. Chemical Aspects 182
B. Three-Dimensional Structure of Head 188
III. Antigenic Structure 194
A. Amino Acid Sequence Variation within Subtypes 194
B. Antigenic Analysis with Antibodies 202
C. Structure of Immune Complexes 206
IV. Summary 209
References 210

Chapter 5

Membrane Insertion and Intracellular Transport of Influenza Virus Glycoproteins

Michael G. Roth, Mary-Jane Gething, and Joe Sambrook

I. Introduction 219
II. Entry of Influenza Glycoproteins into the Exocytic Pathway 225
A. Co-translational Events 225
B. Post-translational Processing in the Endoplasmic Reticulum 231
C. Mutants of Hemagglutinin That Are Defective in Transport from the ER 235
III. Protein Transport between the Endoplasmic Reticulum and the Golgi Apparatus 241
IV. Transport and Processing within the Golgi Apparatus 243
A. Organization of the Golgi Apparatus 243
B. Movement of Influenza Virus Glycoproteins through the Golgi Apparatus 244
C. Processing of Influenza Virus Oligosaccharides 246
D. Role of Glucosylation of Influenza Virus Glycoproteins . 246
V. The *Trans*-Reticular Network 248
A. Sorting of the Hemagglutinin and the Neuraminidase to the Apical Domain of Polarized Epithelial Cells 248

B. Evidence for Proteolytic Processing of Certain Hemagglutinins by Cellular Proteases during Transport to the Cell Surface 251
C. Hemagglutinins Defective in Transport from the Golgi Apparatus to the Cell Surface 251
VI. Transport to the Cell Surface and Insertion into the Plasma Membrane 252
VII. Summary and Perspectives 254
References 255

Chapter 6

Structure of Defective-Interfering RNAs of Influenza Viruses and Their Role in Interference

Debi P. Nayak, Thomas M. Chambers, and Ramesh K. Akkina

I. Introduction 269
II. Nature of the DI Particle Genome 270
A. Generation and Amplification of Influenza Virus DI Particles 270
B. Analysis of DI RNA by PAGE 272
C. Primary Structure of DI RNA 274
III. Generation of Influenza Virus Subgenomic/DI RNA 281
IV. Evolution of DI RNAs 285
V. Replication, Transcription, and Translation of DI RNAs 288
A. Transcription of DI RNAs 288
B. Translation of DI Virus-Specific mRNA 289
VI. Mechanism of Interference 297
A. Effect of DI Virus Particles on the Transcription and Replication of Standard RNAs 298
B. Effect of DI Particles on the Translation of Standard Polypeptides 299
C. Possible Role of DI Virus-Specific Polypeptides in Interference 300
D. Partial Reversal of DI Virus-Mediated Interference with Increasing Concentration of Standard Virus Particles 301
E. Role of DI RNA in Virus Assembly 302
F. Summary 303
VII. Possible Role of Influenza DI Particles in Modulating Viral Pathogenesis 304
VIII. Possible Role of DI Particles in Virus Evolution 308
IX. Summary and Conclusion 310
References 311

Chapter 7

Variation in Influenza Virus Genes: Epidemiological, Pathogenic, and Evolutionary Consequences

Frances I. Smith and Peter Palese

I. Introduction 319
II. Influenza Viruses in Humans 319
III. Homology of Influenza A, B, and C Viruses 322
A. HA/HE Genes 322
B. NP Genes 323
C. M Genes 323
D. NS Genes 324
E. Conclusion 325
IV. Variation in Influenza A Viruses 325
A. Reassortants 325
B. Animal Viruses Become Virulent for Humans 326
C. Re-emergence of Previously Circulating Strains 327
D. Genetic Drift 327
E. Evolution of Influenza A Viruses 334
V. Variation in Influenza C Viruses 336
A. HE Genes 336
B. NS Genes 337
C. Evolution of Influenza C Viruses 337
VI. Variation in Influenza B Viruses 338
A. HA Genes 338
B. NS Genes 338
C. Comparison of Evolutionary Pattern of Influenza A, B, and C Viruses 340
VII. Genetic Basis of Pathogenicity 341
A. Pathogenicity Is Multigenic 341
B. HA Genes 342
C. NA Genes 343
D. M Genes 344
VIII. Comparison of Variation among Different RNA Viruses 345
A. Tissue-Culture Studies 345
B. Variation in Nature of Human RNA Viruses 347
References 350

Chapter 8

Specificity and Function of T Lymphocytes Induced by Influenza A Viruses

Jonathan W. Yewdell and Charles J. Hackett

I. Introduction 361
A. Why Study T-Lymphocyte Responses to Influenza Virus? 361

B. Background Information 362
II. General Properties of Antiviral T-Lymphocyte Responses ... 369
A. Assessing Recognition of Viral Antigens by T Lymphocytes 369
B. Induction of Influenza Virus-Specific T Lymphocytes ... 370
III. Specificity and Function of Anti-influenza Virus T_H 375
A. Specificity of T_H 375
B. Antigen Processing as a Requirement for T_H Recognition of Influenza Virus Components 383
C. Role of T_H in Antiviral Immunity 388
IV. Specificity and Function of Anti-influenza Virus T_C 391
A. Specificity of T_C 391
B. Role of T_C in Antiviral Immunity 410
V. Conclusions 412
References 415

Index 431

CHAPTER 1

Genes and Proteins of the Influenza Viruses

ROBERT A. LAMB

I. INTRODUCTION

This chapter describes the structure of the genes of influenza A, B, and C viruses. Influenza viruses contain a segmented single-stranded RNA genome that has been called negative stranded because the viral messenger RNA (mRNAs) are transcribed from the viral RNA segments. A great deal of new knowledge has been obtained about influenza A, B, and C viruses, since the last major multiauthored reviews of the genetics, molecular biology, and structural biology of influenza viruses (Palese and Kingsbury, 1983). The complete nucleotide sequence of the 8 RNA segments of the influenza A and B viruses has been obtained, and significant progress has been made with the sequencing of the influenza C virus genome. Other major developments include the following:

1. The three-dimensional structure of both major surface antigens, hemagglutinin and neuraminidase, has been determined from X-ray studies of crystallized proteins, and the structure of a neuraminidase–antibody complex has been obtained. In addition, the structure of the influenza virus hemagglutinin complexed with its receptor sialic acid has been elucidated, which may provide a basis for the rational design of antiviral drugs that would block viral attachment to cells.
2. In both influenza A and B viruses, previously unrecognized small integral membrane proteins, M_2 and NB, respectively, have been identified and extensively characterized.

ROBERT A. LAMB • Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois 60208.

3. The influenza A virus N9 neuraminidase has also been found to exhibit hemagglutinating activity.
4. Influenza C virus glycoprotein exhibits both hemagglutinating and neuraminate-O-acetyl esterase activity.

The following sections review the structure of each of the RNA segments of influenza viruses and their encoded proteins. Emphasis is placed on topics unique to this chapter, as later chapters in this volume concern influenza virus replication, the hemagglutinin, the neuraminidase and antigenic variation. Inevitably, there is some overlap, but attempts have been made to minimize it and at the same time will make this chapter an overall survey. Little attempt is made to discuss the history of the disease, the response to infection, the ecology and epidemiology of the disease or the control of influenza through vaccination strategies or antiviral compounds. For these topics, the reader is referred to a recent review by Kilbourne (1987).

A. Structure of the Genome

Influenza virus, when cultured in tissue culture cells or embryonated eggs, has a fairly regular appearance, when negatively stained and visualized in the electron microscope, of particles of 80–120 nm in diameter. The virion contains a lipid envelope containing surface projections or spikes radiating outward. These spikes are of two readily distinguishable types: the rod-shaped hemagglutinin and the mushroom shaped neuraminidase (Kilbourne, 1987). Inside the virus, and observable by thin sectioning of virus or by disrupting particles, are the ribonucleoprotein (RNP) structures, which contain the different RNA segments. The genetic information of influenza A and B viruses is contained in eight segments of single-stranded RNA and for influenza C virus is 7 segments of single-stranded RNA (see Lamb, 1983, and Air and Compans, 1983, for references to the early paper describing these findings). The RNA of the virus is not infectious, and the mRNAs are transcribed from the virion RNA (vRNA) by the virion-associated RNA-dependent RNA transcriptase. Thus, as by convention, mRNA is plus-stranded (Baltimore, 1971) and thus the influenza viruses are known as negative-strand RNA viruses.

The early evidence for a segmented genome of influenza viruses has been extensively reviewed (Lamb, 1983; Lamb and Choppin, 1983); a major step forward in understanding the structure of the influenza virus genome came from the electrophoretic separation of the virion RNAs on polyacrylamide gels containing 6 M urea (Bean and Simpson, 1976; Pons, 1976; Palese and Schulman, 1976; Ritchey *et al.*, 1976; McGeoch *et al.*, 1976) (Fig. 1). The critical study showing that the eight RNA segments of influenza A viruses were distinct was done by two-dimensional

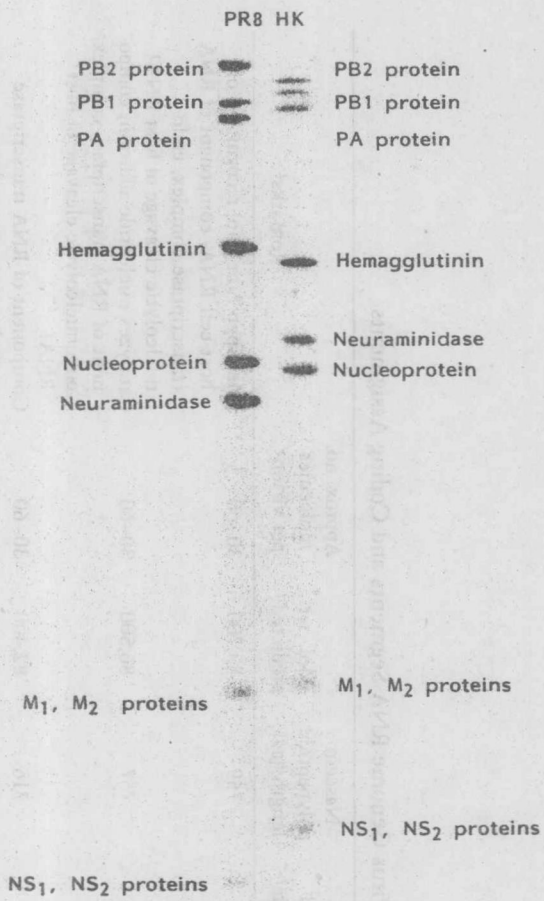

FIGURE 1. The influenza A virus RNA segments indicating their encoded gene products. The RNAs of influenza A/PR/8/34 and A/HK/8/68 viruses (PR8 and HK) were separated on a 2.6% polyacrylamide gel. RNA segments 1–8 are shown from top to bottom of the gel. The gene product assignments are discussed in the text. Under the gel electrophoresis conditions used the migration of the RNAs is dependent on the size as well as the secondary structure of the molecules. From Ritchey *et al.*, (1976) and kindly made available by Dr. Peter Palese, Mount Sinai School of Medicine, New York.

oligonucleotide fingerprinting (McGeoch *et al.*, 1976); this set the stage for a flurry of activity from several laboratories to characterize further the genome RNA segments and to determine their encoded protein products. The assignment of specific RNA segments to virus polypeptides was made in three ways:

1. A method pioneered by Palese, Schulman, and collaborators that depended on the apparent differences between strains in the electrophoretic mobilities of the RNA segments on polyacrylamide gels and the ability to distinguish proteins between strains, either by immunological methods for the hemagglutinin and neuraminidase or by differences in the electrophoretic mobilities of the polypeptides on protein gels [Recombinants were prepared between two parental strains and comparisons made of the mobilities of