List of	Contributors	ΧV
1. The	Evolution of Influenza Viral Genetics - A Perspective.	
By E	E.D.Kilbourne	1
I.	Introduction	1
II.	The Development of Modern Influenza Viral Genetics	2
	A. Early Evidence of Genetic Variation in the Laboratory	2
	B. Application of Formal Genetic Techniques to Studies of	
	Influenza Virus	3
	C. Genetic Markers	3
	D. Development of Plaquing Systems	4
	E. The Use of Conditional Lethal Mutants	5
	F. New Approaches in Influenza Virus Genetics	6
	1. The Biochemical Identification of Viral Gene Products	
	in the Unambiguous Definition of Viral Inheritance	6
	2. Mapping of the Influenza Virus Genome by Correlative	
	Physico-Chemical and Biological Techniques	7
	3. The Application of Molecular Biological Techniques to	
	the Study of Viral Genetic Variation	8
	4. Oligonucleotide Mapping of Viral RNA's	8
	5. Contribution of Protein and RNA Sequencing to Influenza	
	Viral Genetics-Intragenic Mapping	8
III.	Viral Genetics and the Understanding of Viral Virulence and	
	Pathogenicity	9
IV.	Influenza Virus Genetics and the Epidemiology and Evolution	
	of Influenza Viruses (Molecular Epidemiology)	10
	A. Genetic Reassortment in Nature and Its Contribution to the	
	Evolution of New Viruses	10
	B. The Genetics of Minor Variation	11
	C. Influenza A, B, and C Viruses	11

V.	Practical Applications of Influenza Viral Genetics	12
VI.	The Special Genetics of Divided Genome Viruses in Relation	
	to Problems in Influenza	13
VII.	Unsolved Problems in Influenza and Genetic Approaches to	
	Their Solution	14
Refe	rences	14
11010		
	Influenza Virus RNA Segments and Their Encoded Proteins.	
By R	R.A.Lamb	21
I.	Introduction	21
II.	The Influenza Virus Particle: Basic Structure	21
III.	Structure of the Genome	23
	A. Early Evidence for a Segmented Genome	23
	B. The 8 Segments of Influenza Virus RNA	24
	C. Methods for Assigning Gene Functions	26
	D. Sequences at the 5' and 3' Ends of Each RNA Segment Are	
	Common	29
	E. Synthesis of Double-Stranded DNA from Influenza Virus	
	RNA, Cloning and Nucleotide Sequencing	29
IV.	The Influenza Virus RNA Segments	32
	A. RNA Segments 1, 2, and 3: Properties of the Transcriptase	32
	Associated Proteins PB1, PB2, and PA	32
	B. RNA Segment 4: Structure and Function of the Hemag-	32
	glutinin	34
	1. The Structure of RNA Segment 4 Coding for the Hemag-	54
	glutinin	34
	2. The Three-Dimensional Structure of the Hemagglutinin	36
	3. Synthesis of the Hemagglutinin, Cotranslational and Post-	30
	Translational Modifications	37
	4. Cleavage Activation of Infectivity and in Vitro Fusion	38
	C. RNA Segment 5: The Structure of the Nucleocapsid Protein	30
		38
	(NP)	30
	aminidase	39
	E. RNA Segment 7: The Structure and Synthesis of the Mem-	37
	brane Protein (M_1) and Non-Structural Protein (M_2)	41
		41
	F. RNA Segment 8: The Structure and Synthesis of Nonstruc-	44
	tural Proteins NS ₁ and NS ₂	44
	G. Overlapping Coding Regions Using Different Reading Frames	47
A -1-	in Viruses	47
	nowledgements	48
	endix: The Influenza Virus Nucleotide Sequence (A/PR/8/34	40
Strai	n)	48
Kete	rences	57

3. Traf	iscription and Replication of Influenza Viruses. By R. M. Arug.	70
I.	Introduction	70
II.	Viral mRNA Synthesis	71
	A. Priming by Cellular Capped RNAs-Discovery	71
	B. Priming by Cellular Capped RNAs-Mechanism	72
	C. Role of the Three Viral P Proteins in the Steps of Primed	. –
	Transcription	76
	D. Termination of Transcription and Poly (A) Addition	80
	E. Regulation of Viral mRNA Synthesis in the Infected Cell	81
	F. Cellular Site of Viral mRNA Synthesis	82
	G. Role of Other Host Nuclear Functions in Viral mRNA Synthe-	02
	sis—Splicing and Methylation of Internal A Residues	86 ′
III.	Synthesis of Full-Length Transcripts	91
IV.	Synthesis of vRNA (Replication)	93
	erences	94
Kere	rences	77
4. Gen	netic Relatedness of Influenza Viruses (RNA and Protein).	
By C	C. Scholtissek	99
-		
I.	Introduction	99
II.	Genetic Relatedness of Viral RNAs	99
	A. Differences in Migration Rates of the RNA Segments As	
	Revealed by Polyacrylamide Gel Electrophoresis (PAGE)	99
	B. Molecular Hybridization	101
	1. Direct RNA-RNA Hybridization	101
	2. Competitive Hybridization	104
	3. DNA-RNA Hybridization	104
	4. Analysis of Double-Stranded Nuclease S1-Treated Hybrid	
	Molecules by Polyacrylamide Gel Electrophoresis	105
	C. Oligonucleotide Fingerprints	105
	D. Sequencing of RNA Segments	107
	1. Sequencing of ³² P-End Labeled RNAs by Partial Nuclease	
	Digestion,	107
	2. Sequencing of the 3' End of RNAs Using the Dideoxy	
	Method	
	3. Sequencing of Total RNA Segments	
	a) Hemagglutinin Gene	110
	b) Neuraminidase Gene	111
	c) The Three P-Protein Genes	112
	d) The Nucleoprotein Gene	113
	e) The Membrane Protein Gene	113
	f) The Nonstructural Protein Gene	114
III.	Genetic Relatedness of Viral Proteins	116
	A. Differences in Migration Rates of Viral Proteins As Revealed	
	by Polyacrylamide Gel Electrophoresis	116

	B. Tryptic Peptide Mapping	
	C. Direct Sequencing	118
IV.	Concluding Remarks	119
Ackn	owledgements	120
Refer	ences	120
5 Antic	genic Variation Among Type A Influenza Viruses. By R. G. Web-	
	W. G. Laver, and G. M. Air	127
ster, 1	v.G.Laver, and G.M.Aii	12/
I.	Introduction	
II.	Historical	128
III.	Nomenclature	128
IV.		131
V.	The Hemagglutinin (HA)	131
	The Hemagglutinin (HA)	131
	B. Changes in Conformation and Antigenicity of the HA at	
	Low pH	133
		136
	D. Use of Monoclonal Antibodies in the Analysis of Antigenic	
	Drift	138
	E. Sequence Change in the HA of Influenza Virus Variants Se-	
	lected with Monoclonal Antibodies	140
	F. Location of the Antigenic Sites in the 3-D Structure of the	1.0
	HA	141
	G. Sequential Selection of Antigenic Variants	143
	H. Antigenic Drift in the HA of Influenza A Virus from Lower	1 10
	Animals	144
VI.	The Neuraminidase (NA)	146
٧1.	A. Antigenic Variation in the Neuraminidase	148
	B. Selection of NA Variants with Monoclonal Antibodies	150
	C. Antigenic Drift in the Neuraminidases of Influenza A Viruses	150
	from Lower Animals	152
	D. Mechanism of Antigenic Drift	
VII.	· · · · · · · · · · · · · · · · · · ·	
V 11.	Antigenic Shift	
	B. Possible Mechanisms of Shifts in Human Strains	
77777	C. Antigenic Shift in Influenza Viruses from Lower Animals	157 158
	Variation in the Nucleoprotein	
IX.	Variation in the Matrix Protein	158
X.	Variation in the Nonstructural Proteins	159
XI.	Variation in the Polymerase Proteins	160
XII.	What of the Future?	160
	nowledgements	162
Rete:	rences	162

6. Expression of Cloned Influenza Virus Genes. By Ma						
and J. Sambrook	•		•	•		169
I. Introduction						169
II. Expression Vectors						170
III. Expression of Influenza Virus Genes in E. coli .						170
A. Expression of HA						171
B. Expression of NS1 Protein						173
IV. Expression of Influenza Virus Genes in Eukaryotic						
A. Expression of HA in Simian Cells Using Reco						
Viral Vectors						174
B. Preparation of the HA Genes						
C. Construction of Recombinant Genomes						
D. Introduction of Recombinant Genomes into Si						
Production of Virus Stocks						
E. Analysis of the HA Expressed from the SV40-H.	A R	ecc	om	bin	ant	
Vectors						
F. Quantitation of the Amounts of HA Expre	ssec	d 1	froi	m	the	
Recombinant Genomes						
G. Effect of Intervening Sequences in the Rec						
Transcript on the Level of Expression of HA Pr						
H. Expression of the Matrix Gene in Simian C	Cell	s I	Isi	· no	an	101
SV40-M Recombinant Virus	J C 11		001	**6	un	182
I. Transient Expression of Cloned Genes in COS	1 C	:e11	•	•		182
J. Continuous Expression of HA from Genes Inte						
Chromosomes of Eukaryotic Cells						
V. Analysis of the Expression of Mutant HA Proteins						
A. The Signal-Minus HA Is a Non-Glycosylate						
Protein						
B. Removal of the C-Terminal Hydrophobic Sequ	· ien	Ce	Сс	•	erte	103
HA into a Secreted Protein						
C. Future Prospects						
References						
References	•	•	•	•	•	170
7. Mutants of Influenza Virus. By B. W. J. Mahy			•			192
I. Introduction						192
II. Characterization of Virus Mutants						193
A. Influenza Virus-Cell Systems						194
B. Nature of the Virus Population						194
C. Natural Mutation Rate						195
D. Induction of Mutants						196
1. Mutagens						196
E. Leak and Reversion						197
III. Temperature-Sensitive Mutants						199
A. Genetic Interaction and Classification of Muta:			•			100

	1. Cambridge Mutants	201
	2. New York-Tokyo Mutants	202
	3. Giessen Mutants	202
	4. Moscow Mutants	204
	5. Bethesda Mutants	204
	6. Summary	205
	C. Phenotypic Analysis of Temperature Sensitive Mutants	206
	1. Assignment of Polypeptides to RNA Segments	206
	2. Mutants with ts Lesions in RNA Segment 1	208
	a) Virion RNA Transcriptase Activity	208
	b) RNA-Dependent RNA Polymerase Activity of Infected	
	Cell Extracts	210
		211
		214
	, , , , , , ,	215
	3. Mutants with ts Lesions in RNA Segment 2	217
	· · · · · · · · · · · · · · · · · · ·	218
	a) Virion Transcriptase Activity	210
	,	218
	Cell Extracts	219
	, 1	220
	, , , , , ,	
	e) Summary	
	4. Mutants with ts Lesions in RNA Segment 3	
	a) Virion Transcriptase Activity	223
	b) RNA-Dependent RNA Polymerase Activity of Infected	222
	Cell Extracts	223
	c) Virus-Specific RNA Synthesis in vivo	
	, , , , , , , ,	224
	e) Summary	224
	5. Mutants with ts Lesions in RNA Segment 4	226
	6. Mutants with ts Lesions in the RNA Segments Encoding	
	the Nucleoprotein	227
	-,	227
	-,1	228
	c) Virus-Specific Polypeptide Synthesis	
	d) Summary	230
	7. Mutants with ts Lesions in the RNA Segment Encoding the	
	Neuraminidase	230
	8. Mutants with ts Lesions in RNA Segment 7	231
	9. Mutants with ts Lesions in RNA Segment 8	232
	D. Temperature-Sensitive Mutant-Derived Vaccines	235
IV.	Cold-Adapted Mutants	235
V.		236
	A. Non-Conditional Host Range Mutants	237
	B. Conditional Host Range Mutants	238
	C. Conclusions	

	V1.	Amantadine-Resistant Mutants	
	VII.		
		owledgements	
	Refer	ences	242
8.	The	Structure of Influenza Virus Defective Interfering (DI) RNAs	
٠.		Their Progenitor Genes. By D. P. Nayak and N. Sivasubramanian	255
		- 1.03 - 1.08 - 1.01 - 1.01 - 1.01 - 1.01 - 1.01 - 1.01 - 1.01 - 1.01 - 1.01 - 1.01 - 1.01 - 1.01 - 1.01 - 1.01	
	I.	Introduction	255
	II.	Properties of Influenza DI Particles	255
	III.	RNA of Influenza Virus DI Particles	
	IV.	Structure of Polymerase Genes and Polymerase Proteins	
	- , ,	A. Nucleotide Sequence of Polymerase Genes	
		B. Primary Structure of PB1 and PB2 Proteins	
		C. Predicted Secondary Structures of PB1 and PB2 Proteins	
	V.	Structure of DI RNAs	
	٧.	A. Classes of DI RNAs	
		B. Complete Nucleotide Sequences of DI RNAs	
	3.71		
	VI.	Generation of DI RNAs from the Progenitor RNAs	
	VII.	Transcription of Influenza Virus DI Viruses	
		Interference by Influenza DI Viruses	
	IX.	Conclusion	
		owledgement	
	Refe	rences	276
	Refe	rences	276
9			
9	. Influ	nenza B and Influenza C Viruses. By G. M. Air and R. W. Compans	280
9	. Influ I.	nenza B and Influenza C Viruses. By G. M. Air and R. W. Compans Introduction	280 280
9	. Influ	Introduction	280 280 280
9	. Influ I.	Introduction	280 280 280 280
9	. Influ I.	Introduction	280 280 280 280
9	. Influ I.	Introduction	280 280 280 280 283
9	. Influ I.	Introduction	280 280 280 280 283 283
9	. Influ I.	Introduction	280 280 280 280 283 283 285
9	. Influ I.	Introduction	280 280 280 280 283 283 285 288
9	. Influ I.	Introduction	280 280 280 280 283 283 285 288 289
9	. Influ I.	Introduction	280 280 280 280 283 283 285 288 289 290
9	. Influ I.	Introduction	280 280 280 283 283 285 288 289 290 290
9	. Influ I.	Introduction	280 280 280 283 283 285 288 289 290 290
9	. Influ I. II.	Introduction. Influenza B Virus. A. Influenza B RNA Species. B. Influenza B Proteins. 1. Hemagglutinin. 2. Neuraminidase. 3. Matrix Protein. 4. The Non-Structural Proteins. 5. The P Proteins. C. Replication of Influenza B Virus D. Epidemiology. E. Antigenic Variation in Influenza B Viruses.	280 280 280 283 283 285 288 289 290 291 292
9	. Influ I.	Introduction. Influenza B Virus. A. Influenza B RNA Species. B. Influenza B Proteins. 1. Hemagglutinin. 2. Neuraminidase. 3. Matrix Protein. 4. The Non-Structural Proteins. 5. The P Proteins. C. Replication of Influenza B Virus D. Epidemiology E. Antigenic Variation in Influenza B Viruses. Influenza C Virus.	280 280 280 283 283 285 288 289 290 290 291 292 293
9	. Influ I. II.	Introduction. Influenza B Virus. A. Influenza B RNA Species. B. Influenza B Proteins. 1. Hemagglutinin. 2. Neuraminidase. 3. Matrix Protein. 4. The Non-Structural Proteins. 5. The P Proteins. C. Replication of Influenza B Virus D. Epidemiology. E. Antigenic Variation in Influenza B Viruses. Influenza C Virus. A. Virus Structure.	280 280 280 283 283 285 288 289 290 290 291 292 293 293
9	. Influ I. II.	Introduction. Influenza B Virus. A. Influenza B RNA Species. B. Influenza B Proteins. 1. Hemagglutinin. 2. Neuraminidase. 3. Matrix Protein. 4. The Non-Structural Proteins. 5. The P Proteins. C. Replication of Influenza B Virus D. Epidemiology E. Antigenic Variation in Influenza B Viruses. Influenza C Virus. A. Virus Structure 1. Morphology of Virions.	280 280 280 283 283 285 288 289 290 291 292 293 293 293
9	. Influ I. II.	Introduction. Influenza B Virus. A. Influenza B RNA Species. B. Influenza B Proteins. 1. Hemagglutinin. 2. Neuraminidase. 3. Matrix Protein. 4. The Non-Structural Proteins. 5. The P Proteins. C. Replication of Influenza B Virus D. Epidemiology. E. Antigenic Variation in Influenza B Viruses. Influenza C Virus. A. Virus Structure 1. Morphology of Virions. 2. Viral RNA Species	280 280 280 283 283 285 288 290 290 291 292 293 293 293 294
9	. Influ I. II.	Introduction. Influenza B Virus. A. Influenza B RNA Species. B. Influenza B Proteins. 1. Hemagglutinin. 2. Neuraminidase. 3. Matrix Protein. 4. The Non-Structural Proteins. 5. The P Proteins. C. Replication of Influenza B Virus D. Epidemiology. E. Antigenic Variation in Influenza B Viruses. Influenza C Virus. A. Virus Structure 1. Morphology of Virions. 2. Viral RNA Species 3. Viral Polypeptides.	280 280 280 283 283 285 288 290 290 291 292 293 293 293 294 295
9	. Influ I. II.	Introduction. Influenza B Virus. A. Influenza B RNA Species. B. Influenza B Proteins. 1. Hemagglutinin. 2. Neuraminidase. 3. Matrix Protein. 4. The Non-Structural Proteins. 5. The P Proteins. C. Replication of Influenza B Virus D. Epidemiology. E. Antigenic Variation in Influenza B Viruses. Influenza C Virus. A. Virus Structure 1. Morphology of Virions. 2. Viral RNA Species	280 280 280 283 283 285 288 289 290 291 292 293 293 293 294 295 297

		B. Influenza C Virus Replication														
		C. Genetics and Epidemiology .														
	Ackn	owledgements														300
	Refer	ences														300
10.		-Determined Differences in														
	Virus	Infections. By J. L. Schulman.			•			•	•	•		•				305
	Ī.	Introduction														305
	II.	Methods														
	III.	Hemagglutinin-Related Difference														
	IV.	Virulence in Chickens														
	V.	Neurovirulence in Mice														
	VI.	Virulence in Other Experimenta														
	V 1.	A. Ferrets														
		B. Rats														
		C. Mice														
	VII.			41.	•	C	•	•	•	•	•	•	•	•	•	214
	٧11.															
		A. Cell Culture														
		B. Yield in Embryonated Eggs														
		C. Sensitivity to Amantadine.														
		Virulence in Man														
	IX.	Summary														
	Refer	ences	•	•	•	•	•	•	•	•	٠	•	٠	•	٠	317
	37.1	1 7 1 1 1 6 7 7			,				ח	n	ת	. ,			,	
11.		cular Epidemiology of Influ														221
	J. F. 1	oung	•	•	•	•	•	٠	٠	•	•	•	•	•	٠	321
	ĭ.	Introduction														321
	II.	Influenza A, B, and C Viruses a														
	III.	Influenza in the 20th Century														
	IV.	Surveillance of Influenza Viruse														
	V.	The 1977 H1N1 Viruses														
	v. VI.	Molecular Epidemiology of Influ														
		Molecular Epidemiology of Infid	1611	za	111	. A	1111 T.	וומו - ח	5	•	•		_ T	?:_1		323
	VII.	Mechanisms Contributing to V														
		Strains														
		A. Point Mutations														
		B. Recombination (Reassortmen														
		C. Deletions/Insertions														
		D. Recycling of Genes														
		Outlook for the Future														
	Refe	rences			•				•				•		٠	334
	Subj	ect Index														337