Reverse Genetics of RNA Viruses

Reverse Genetics of RNA Viruses

Applications and Perspectives

Edited by

Anne Bridgen

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First Impression 2013

I would like to dedicate this book to Professor Sir Kenneth Murray, FRS, FRSE, for his mentoring during the course of my PhD and his introduction to the powerful world of molecular biology.

Ken, you were an inspiration in the way in which you searched out important issues in science and tackled them, no matter how insurmountable the obstacles. Your groundbreaking work on the manipulation of hepatitis B virus and early development of an effective and safe vaccine has been much of the inspiration to my work in this field, and I thank you for this.

Contents

List of contributors **xi**

Acknowledgements **xiii**

1 Introduction **1**
Anne Bridgen

1.1 Background 1
1.2 Reverse genetics for different classes of genome 2
1.3 Methodology 5
1.4 Difficulties in establishing a reverse genetics system 11
1.5 Recent developments 13
1.6 Are there any boundaries for conducting reverse genetics? 13
References 15

Part I Positive sense RNA viruses **25**

2 Coronavirus reverse genetics **27**
Maria Armesto, Kirsten Bentley, Erica Bickerton, Sarah Keep and Paul Britton

2.1 The *Coronavirinae* 27
2.2 Infectious bronchitis 28
2.3 Coronavirus genome organisation 29
2.4 The coronavirus replication cycle 30
2.5 Development of reverse genetics system for coronaviruses including IBV 33
2.6 Reverse genetics system for IBV 37
2.7 Reverse genetics systems for the modification of coronavirus genomes 40
2.8 Using coronavirus reverse genetics systems for gene delivery 49
Acknowledgements 51
References 51

3 Reverse genetic tools to study hepatitis C virus **64**
Alexander Ploss

3.1 Introduction: hepatitis C 64
3.2 Hepatitis C virus 65
3.3 Construction of infectious clones for hepatitis C virus 68
3.4 Study of HCV RNA replication in cell culture systems 68

3.5 Use of HCV replicons to study viral replication 70
3.6 Utility of replicons for drug screening 71
3.7 Development of the infectious cell culture systems for HCV 71
3.8 Construction of intergenotypic viral chimeras 72
3.9 Non-JFH1 derived genomes 74
3.10 Cell lines that support HCV replication 74
3.11 Study of HCV in physiologically more relevant cell culture systems 75
3.12 Animal models for HCV infection 76
3.13 Reverse genetics of clinically relevant HCV genotypes *in vivo* 77
3.14 Conclusion 78
Acknowledgments 78
References 78

4 Calicivirus reverse genetics 91
Ian Goodfellow

4.1 Introduction 91
4.2 Feline calicivirus 93
4.3 Murine norovirus 97
4.4 Porcine enteric calicivirus 103
4.5 Rabbit haemorrhagic disease virus 104
4.6 Human norovirus 104
4.7 Conclusion 106
Acknowledgements 107
References 107

Part II Negative sense RNA viruses 113

5 Reverse genetics of rhabdoviruses 115
Alexander Ghanem and Karl-Klaus Conzelmann

5.1 Introduction: the *Rhabdoviridae* family 115
5.2 Rhabdovirus reverse genetics 121
5.3 Applications and examples 132
5.4 Conclusion 137
Acknowledgements 137
References 137

6 Modification of measles virus and application to pathogenesis studies 150
Linda J. Rennick and W. Paul Duprex

6.1 Introduction 150
6.2 Measles: the disease 150
6.3 Measles: the infectious agent 151
6.4 RNA synthesis: a tail of two processes 154
6.5 Transcription: starting, stopping, dropping off or starting again 154
6.6 From transcription to replication: the elusive switch 155
6.7 Getting in and getting out 157

6.8 Measles virus: reverse genetics 158
6.9 Future perspectives 181
Acknowledgements 182
References 182

7 Bunyavirus reverse genetics and applications to studying interactions with host cells 200
Richard M. Elliott

7.1 Introduction: the family *Bunyaviridae* 200
7.2 Bunyavirus replication 201
7.3 History of bunyavirus reverse genetics 203
7.4 Minigenome systems for bunyaviruses 205
7.5 Virus-like particle production 207
7.6 Rescue systems for bunyaviruses 208
7.7 Application of reverse genetics to study bunyavirus replication 208
7.8 Outlook 215
References 216

8 Using reverse genetics to improve influenza vaccines 224
Ruth A. Elderfield, Lorian C.S. Hartgroves and Wendy S. Barclay

8.1 Introduction 224
8.2 Influenza vaccines 227
8.3 The use of reverse genetics to generate recombinant influenza A, B and C viruses 229
8.4 Using reverse genetics technology for generation of pandemic virus vaccine 232
8.5 Other strategies for generating live attenuated vaccines based on viruses engineered by reverse genetics 235
8.6 Strategies to improve the safety or yield of influenza vaccines 238
8.7 Improvements to the PR8 high growth strain 239
8.8 Improving the immunogenicity by engineering recombinant viruses that express cytokine genes 240
8.9 Novel species-specific attenuation that takes advantage of microRNAs 240
8.10 Conclusion 241
References 241

Part III Double-stranded RNA viruses 251

9 Bluetongue virus reverse genetics 253
Mark Boyce

9.1 Introduction to Bluetongue virus 253
9.2 Bluetongue virus replication 254
9.3 Reverse genetics 260
9.4 Uses of reverse genetics in orbivirus research 271
9.5 Future perspectives 278
References 281

10 Genetic modification in mammalian orthoreoviruses **289**
Sanne K. van den Hengel, Iris J.C. Dautzenberg, Diana J.M. van den Wollenberg, Peter A.E. Sillevis Smitt and Rob C. Hoeben

10.1 Introduction 289
10.2 Forward-genetics in orthoreoviruses 296
10.3 Reovirus/cell interactions 297
10.4 Reverse-genetics in orthoreoviruses 301
10.5 Reovirus as an oncolytic agent 306
10.6 Conclusion 308
References 309

Part IV Recent and future developments **319**

11 Reverse genetics and quasispecies **321**
Antonio V. Bordería and Marco Vignuzzi

11.1 Definition of quasispecies and evidence 321
11.2 Reverse genetics and RNA virus population heterogeneity: consensus is always a compromise 328
11.3 Examples of the use of the theory to disable or manipulate the quasispecies under controlled environments 333
11.4 Future prospects of virus population genetics and reverse genetics 339
11.5 Conclusion 341
References 342

12 Summary and perspectives **350**
Anne Bridgen

12.1 Introduction 350
12.2 Analysis of the role of specific non-coding sequence motifs involved in replication, transcription, polyadenylation and packaging 351
12.3 Analysis of the roles of viral proteins 352
12.4 Analysis of virus–host interactions at a global level 353
12.5 Understanding the basis of pathogenicity 354
12.6 Real-time virus imaging *in vitro* and *in vivo* 355
12.7 Structure-function analysis of viruses and viral domains 356
12.8 Vaccine generation 357
12.9 Drug development 359
12.10 Gene delivery and knock-out in plant cells including virus-induced gene silencing (VIGS) 361
12.11 Gene delivery in arthropod and mammalian cells 362
12.12 Development of oncolytic virus and adaptation to this purpose 363
12.13 Personal highlights and future directions 364
References 366

Index **375**

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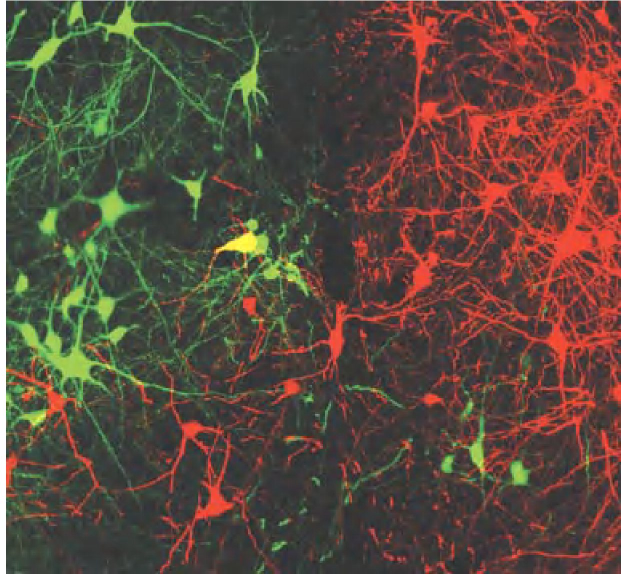

Plate 1 Examples of monosynaptic tracing of neuronal connections with RABV. Interneurones of the spinal cord which are directly connected to motor neurones of the left (green) and right (red) quadriceps muscle. The yellow interneurone is connected to both right and left motoneurones. G gene-deficient RABV expressing GFP (SADΔG-eGFP) or RFP (SADΔG-RFP) were injected into the right or left muscle, respectively, and there infect motor neurones expressing RABV G from an AAV vector. The G protein mediates a single transsynaptic spread of RABV to the postsynaptic interneurones.
Source: Kindly provided by Anna Stepien and Silvia Arber, University of Basel.

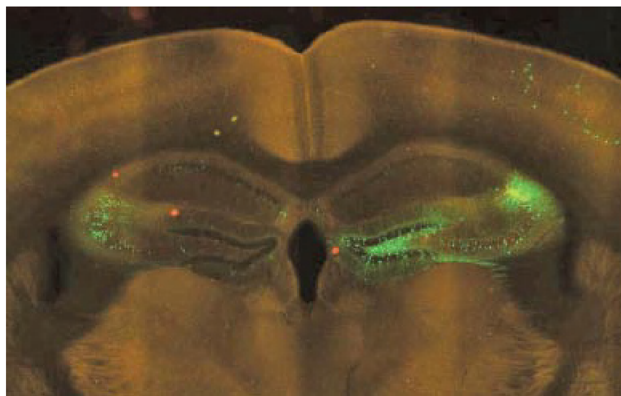

Plate 2 Direct connections of right and left hippocampal neurones in the mouse brain. The CA3 region in the right hippocampus (yellow) was injected with an AAV vector expressing TVA, td-tomato, and RABV G protein. Subsequent selective infection of TVA-expressing neurones with the RABV SADΔG-eGFP pseudotyped with EnvA is indicated by yellow. Green staining indicates neurones infected by transsynaptic spread of SADΔG-eGFP and reveals direct connections between left and right hemispheres.
Source: Kindly provided by Martin Schwarz, MPIMF Heidelberg.

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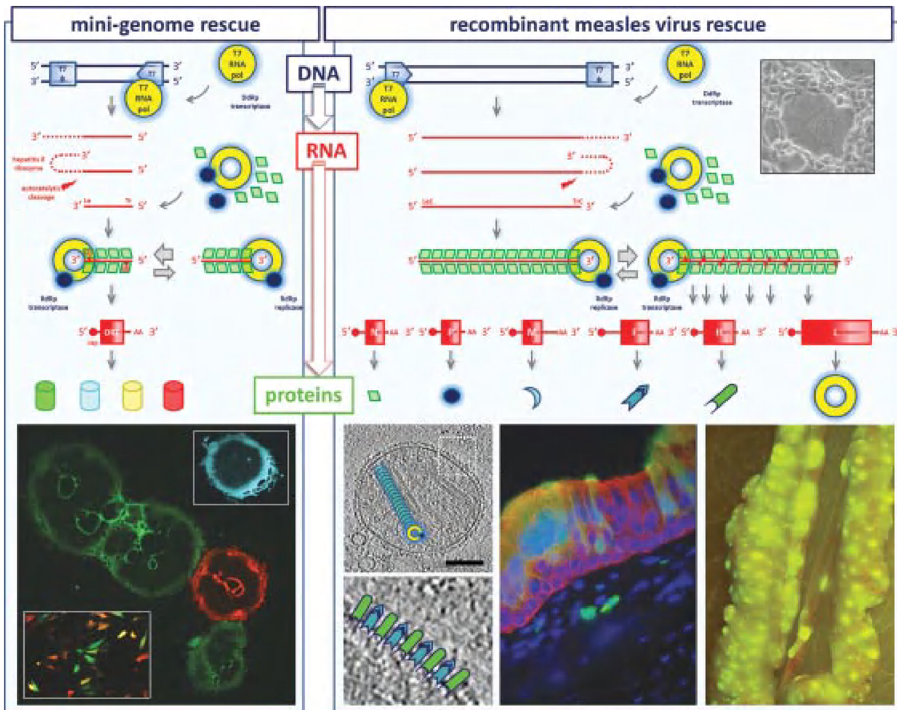

Plate 3 Schematic representations of the mini-genome (left) and recombinant measles virus (right) rescue systems used to study transcription and replication and to generate rMVs respectively. T7 bacteriophage DNA-dependent RNA polymerase (DdRp) is supplied by a recombinant host-range adapted vaccinia virus (MVA-T7). The transcriptase recognises the T7 promoter engineered into the plasmid backbone to generate either a negative sensed mini-genome RNA or a full-length genome positive sensed transcript. Presence of T7 terminators (T7ϕ) leads to the detachment of the transcriptase from the DNA template. Nascent RNA transcripts contain a hepatitis δ ribozyme at the 3′ end (dashed red line). Formation of the secondary structure leads to the autocatalytic cleavage of the T7 transcript *in cis*. This generates minigenome or genome length transcripts which conform to the 'rule of six'. The minigenome is flanked by leader (Le) and trailer (Tr) sequences and the antigenome is flanked by leader complement (LeC) and trailer complement (TrC) sequences. Co-transfection of three helper plasmids which also contain T7 promoters and the open reading frames encoding the virus N (green rhombus), P (blue circle) and L (yellow ring) proteins allows the formation of either a negative sensed minigenomic (−)RNP or a positive sensed antigenomic (+)RNP. The L and P proteins function as a transcriptase on the minigenomic (−)RNP producing a single capped (red circle) and polyadenylated (AA) mRNA containing the open reading frame (ORF) of a reporter protein such as enhanced green fluorescent protein (green barrel), enhanced cyan fluorescent protein (cyan barrel), enhanced yellow fluorescent protein (yellow barrel) or HcRed (red barrel). The L and P proteins also function as a replicase generating (+)RNP minigenomes from the (−)RNP template. These (+)RNP minigenomes are in turn replicated to produce additional (−)RNP minigenomes. Expression of the fluorescent proteins is detected by UV microscopy in single cells (inset) when the system is driven exclusively by cotransfected plasmids or in multinucleated syncytia when the minigenome replication/transcription assay is driven by a superinfecting MV. In recombinant measles virus rescue the L and P proteins initially act as a replicase using the positive sensed antigenomic (+)RNP to generate the negative sensed genomic (−)RNP. This is the basic unit of infectivity of MV and the L and P proteins function as a transcriptase on this full-length (−)RNP producing six capped and polyadenylated mRNAs containing the N, P, M (cyan crescent), F (blue arrow), H (green bullet) and L gene ORFs. Translation of these proteins allows assembly of virions at the plasma membrane. When virions are examined by electron cryotomography the M protein can be seen coating the RNP (0.8 nm thick slice from a tomogram, scale bar 100 nm). The herringbone structure of the (−)RNP is clearly visible within the virion, a schematic (−)RNP is overlaid for comparison (left panel). A fringe of spikes of the F and H fusion complex decorates the membrane of the virion; these are represented schematically at a higher magnification on the same tomograph. Recombinant MVs based on clinical isolates expressing fluorescent proteins from additional transcription units have been invaluable in illuminating viral pathogenesis. These viruses permit the microscopic imaging of virus infected cells with unprecedented levels of sensitivity, for example in epithelia (center panel). They also allow macroscopic imaging and targeted pathology to be performed at the time of necropsy, for example in the gut associated lymphoid tissue of a macaque (right panel).

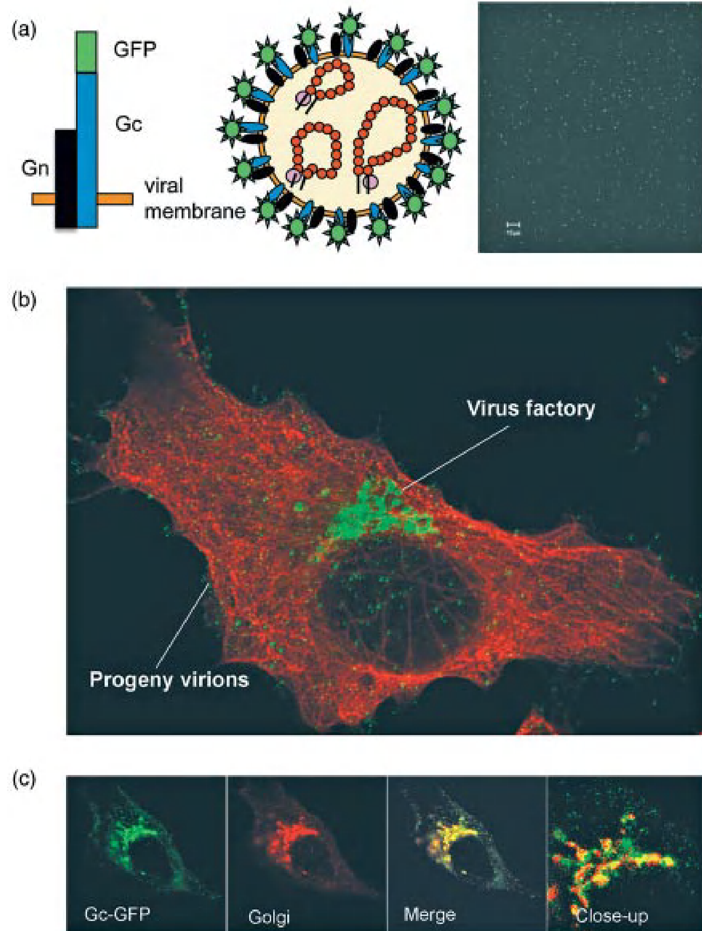

Plate 4 Recombinant Bunyamwera virus expressing GFP-Gc fusion protein. (a) Schematics of the chimeric glycoprotein and recombinant virus (rBUNGc-eGFP) are shown on the left. On the right is supernatant fluid from infected cells examined under UV light showing autofluoresent virus particles. (b) BSR-T7/5 cells were infected for 8 h with rBUNGc-eGFP at an MOI of 1 PFU/ cell, fixed and co-stained with anti-tubulin antibody (in red). The virus factory in the Golgi region of the cell and autofluorescent progeny virus particles are indicated. (c) Detail of virus budding at the Golgi. BSRT7/5 cells were infected with rBUNGc-eGFP and co-stained with antibodies to the Golgi marker GM130 (in red). Colocalization between Gc proteins and the Golgi protein are shown in yellow in the merged image, and the enlarged image shows budding virions. *Source*: Adapted from Shi *et al*., (2010). Copyright © American Society for Microbiology. *Journal of Virology,* Vol. 84, 2010, p. 8460–8469. doi:10.1128/JVI.00902-10.

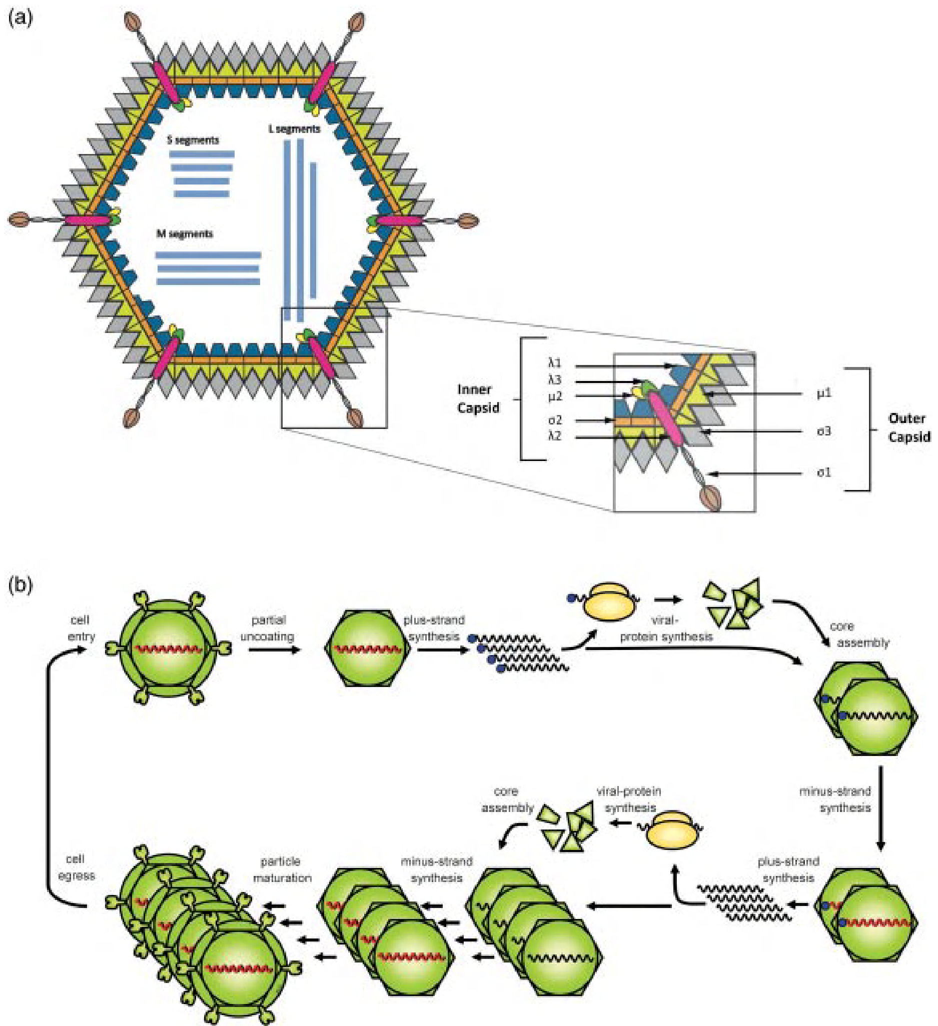

Plate 5 (a) Schematic representation of the mammalian orthoreovirus. The virus contains a non-enveloped icosahedral capsid, containing 10 dsRNA segments. These encode the structural proteins: five proteins comprise the inner capsid, and the three others form the outer capsid. The positions of the various capsid components are indicated. (b) Schematic representation of the reovirus' genome replication. After cell entry the viral particle is partially uncoated, and penetrates the endosomal membrane. In the cytoplasm the primary transcription process yields capped plus-strand RNA molecules, which are translated and can associate with the newly assembled cores. In the cores these transcripts serve as templates for minus-strand synthesis yielding double-stranded RNA. Subsequently the secondary transcription process yields uncapped transcripts which are translated and associate with the new core particles. Minus-strand synthesis proceeds to yield double-stranded RNA genome segments. The particles mature and egress from the cells. In the figure only one of the genome segments is drawn.