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Preface

In this book we aimed to describe a variety of techniques that reflect the wide range of research currently being performed in the field of coronavirology. However, most of the techniques described are also applicable to a wide variety of other virology fields, so we hope that this book will have wider appeal. As such, we have started this book with an overview chapter of current understanding of coronavirus replication and pathogenesis to introduce nonspecialist readers to the field.

Since the emergence of SARS-Coronavirus in 2003, numerous new coronaviruses have been identified. The emergence of MERS-Coronavirus in 2012 and the continued occurrence of human cases highlight the importance of techniques to verify the presence of coronaviruses in a sample as well as identify new coronaviruses that may pose a potential threat to the health of both humans and livestock. As such, chapters have been chosen to describe identification, diagnosis, and study of evolution of coronaviruses.

To allow the study of viruses, propagation and quantification of virus is essential. Therefore, we have included chapters describing preparation of cells and organ cultures useful in propagating coronaviruses and titration techniques. In addition, several techniques for analyzing virus function require purification of virus, so purification protocols suitable for different downstream techniques have been included.

The ability to reverse engineer virus genomes and recover recombinant viruses with defined mutations is invaluable in the progression of understanding the mechanisms for virus pathogenicity, viral protein and RNA function and understanding virus-host interactions. Therefore, chapters describing two commonly used reverse genetics techniques for coronaviruses are included.

A key step in virus replication is attachment to and entry into the host cell. Techniques detailing identification of cellular receptors, binding profiles of viral attachment proteins, and virus-cell fusion are described.

Finally, a major area of coronavirus research currently is the interaction between the virus and the host cell to gain insight into requirements of the virus to enable replication but also how the host cell responds to virus infection. Understanding these processes is vital in enabling future control of virus replication with antiviral therapeutics or prevention through vaccination. Therefore, several chapters have been included covering a broad spectrum of techniques to identify virus-host protein-protein interactions, confirm the functional role of these proteins in virus replication, study host cell responses through genome-wide or pathway-specific approaches, and visualise virus replication complexes.

We would like to thank the authors who have contributed to this book for the time they have taken to prepare detailed methods as well as provide practical hints and tips that are often essential to get a new working protocol.

Compton, UK

Helena Jane Maier
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Chapter 1

Coronaviruses: An Overview of Their Replication and Pathogenesis

Anthony R. Fehr and Stanley Perlman

Abstract

Coronaviruses (CoVs), enveloped positive-sense RNA viruses, are characterized by club-like spikes that project from their surface, an unusually large RNA genome, and a unique replication strategy. Coronaviruses cause a variety of diseases in mammals and birds ranging from enteritis in cows and pigs and upper respiratory disease in chickens to potentially lethal human respiratory infections. Here we provide a brief introduction to coronaviruses discussing their replication and pathogenicity, and current prevention and treatment strategies. We also discuss the outbreaks of the highly pathogenic Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and the recently identified Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV).

Key words Nidovirales, Coronavirus, Positive-sense RNA viruses, SARS-CoV, MERS-CoV

1 Classification

Coronaviruses (CoVs) are the largest group of viruses belonging to the *Nidovirales* order, which includes *Coronaviridae*, *Arteriviridae*, *Mesoniviridae*, and *Roniviridae* families. The *Coronavirinae* comprise one of two subfamilies in the *Coronaviridae* family, with the other being the *Torovirinae*. The *Coronavirinae* are further subdivided into four genera, the alpha, beta, gamma, and delta coronaviruses. The viruses were initially sorted into these genera based on serology but are now divided by phylogenetic clustering.

All viruses in the *Nidovirales* order are enveloped, non-segmented positive-sense RNA viruses. They all contain very large genomes for RNA viruses, with some viruses having the largest identified RNA genomes, containing up to 33.5 kilobase (kb) genomes. Other common features within the *Nidovirales* order include: (1) a highly conserved genomic organization, with a large replicase gene preceding structural and accessory genes; (2) expression of many non-structural genes by ribosomal

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frameshifting; (3) several unique or unusual enzymatic activities encoded within the large replicase–transcriptase polyprotein; and (4) expression of downstream genes by synthesis of 3′ nested subgenomic mRNAs. In fact, the *Nidovirales* order name is derived from these nested 3′ mRNAs as *nido* is Latin for "nest." The major differences within the Nidovirus families are in the number, type, and sizes of the structural proteins. These differences cause significant alterations in the structure and morphology of the nucleocapsids and virions.

2 Genomic Organization

Coronaviruses contain a non-segmented, positive-sense RNA genome of ~30 kb. The genome contains a 5′ cap structure along with a 3′ poly (A) tail, allowing it to act as an mRNA for translation of the replicase polyproteins. The replicase gene encoding the nonstructural proteins (nsps) occupies two-thirds of the genome, about 20 kb, as opposed to the structural and accessory proteins, which make up only about 10 kb of the viral genome. The 5′ end of the genome contains a leader sequence and untranslated region (UTR) that contains multiple stem loop structures required for RNA replication and transcription. Additionally, at the beginning of each structural or accessory gene are transcriptional regulatory sequences (TRSs) that are required for expression of each of these genes (*see* Subheading 4.3 on RNA replication). The 3′ UTR also contains RNA structures required for replication and synthesis of viral RNA. The organization of the coronavirus genome is 5′-leader-UTR-replicase-S (Spike)-E (Envelope)-M (Membrane)-N (Nucleocapsid)-3′ UTR-poly (A) tail with accessory genes interspersed within the structural genes at the 3′ end of the genome (Fig. 1). The accessory proteins are almost exclusively nonessential for replication in tissue culture; however, some have been shown to have important roles in viral pathogenesis [1].

3 Virion Structure

Coronavirus virions are spherical with diameters of approximately 125 nm as depicted in recent studies by cryo-electron tomography and cryo-electron microscopy [2, 3]. The most prominent feature of coronaviruses is the club-shaped spike projections emanating from the surface of the virion. These spikes are a defining feature of the virion and give them the appearance of a solar corona, prompting the name, coronaviruses. Within the envelope of the virion is the nucleocapsid. Coronaviruses have helically symmetrical nucleocapsids, which is uncommon among positive-sense RNA viruses, but far more common for negative-sense RNA viruses.