CHAPTER 1

The *Coronaviridae*

An Introduction

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I. INTRODUCTION

The *Coronaviridae* are a group of enveloped, positive-strand RNA viruses with nonsegmented genomes of about 30,000 nucleotides. The family comprises two genera, coronavirus and torovirus, which share similarities in the organization and expression of their genomes and the structure of the viral gene products. Viruses in the two genera have, however, only limited sequence similarity and display; for example, distinctly different nucleocapsid morphologies.

In this introductory chapter, I shall briefly discuss the classification of the *Coronaviridae*, the organization of corona- and torovirus genomes, and the morphology and physicochemical properties of the virus particles. The molecular aspects of coronavirus and torovirus biology are discussed extensively in Chapters 2 to 12, and Chapters 13 to 19 deal with the biological aspects of coronavirus and torovirus infections.

II. CLASSIFICATION

Viruses that are classified in the *Coronaviridae* fulfill the following criteria:

- A positive-strand, nonsegmented genome of about 30,000 nucleotides. The genome contains an unusually large RNA polymerase gene (about

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20,000 nucleotides), a large surface glycoprotein gene, an integral membrane protein gene, and a nucleocapsid protein gene. These genes are arranged on the genome in a specific order (as described above in the 5′ → 3′ direction) but may be interspersed with additional genes encoding further structural or nonstructural proteins.

- A virion envelope bearing pronounced surface projections. These projections are composed of the large surface glycoprotein (M_r = ~200 kDa) that characteristically exhibits a coiled-coil structure in the carboxy-terminal, membrane-anchoring half.
- An integral membrane protein (M_r = ~25 kDa). This protein characteristically has three membrane-spanning regions in the amino-terminal half.
- A 3′ coterminal set of four or more intracellular subgenomic mRNAs. Only the open reading frames (ORFs) contained within the 5′ unique region of each mRNA (i.e., the region not found in the next smallest mRNA) are expressed as protein.
- An RNA polymerase gene composed of two overlapping ORFs. Expression of the downstream ORF is mediated by (−1) ribosomal frameshifting.

The features that distinguish coronaviruses and toroviruses are:

- The size of the nucleocapsid protein (M_r = ~60 kDa and ~18 kDa, respectively) and the shape of the helical nucleocapsid structure (extended or tubular, respectively).
- The absence of a leader sequence at the 5′ end of torovirus mRNAs.
- With some limited exceptions, a lack of sequence similarity in the coronavirus and torovirus gene products.

At the present time 13 viruses are classified as species in the *Coronaviridae* (Cavanagh *et al.*, 1994). These are listed with their acronyms and natural hosts in Table I.

The prototype of the coronavirus genus is avian infectious bronchitis virus. The name "coronavirus" is derived from the solar corona-like (L. *corona* = crown) appearance of virus particles in negatively stained electron micrographs. The prototype of the torovirus genus is Berne virus (BEV) and the name "torovirus" is derived from the curved tubular (L. *torus* = lowest convex molding in the base of a column) morphology of the nucleocapsid structure.

In addition to the viruses listed in Table I, two viruses, rat coronavirus (RCV) and rabbit coronavirus (RbCV), are considered as tentative species of the coronavirus genus. Three more viruses, feline enteric coronavirus (FECV), sialoacryoadenitis virus (SADV), and porcine respiratory coronavirus (PRCV), are often discussed in the literature as coronavirus species, but the available data suggest that they could be equally well considered as variants of feline infectious peritonitis virus (FIPV), RCV, and transmissible gastroenteritis virus (TGEV), respectively.

In relation to the torovirus genus, there are reports of an enveloped virus in the stools of patients with gastroenteritis that resembles Breda viruses (Beards *et al.*, 1984). However, this virus is not yet recognized as a member of the genus.

TABLE I. *Coronaviridae*

Natural host	Virus	Acronym
Coronavirus		
Chicken	Avian infectious bronchitis virus	IBV
Cattle	Bovine coronavirus	BCV
Dog	Canine coronavirus	CCV
Man	Human coronavirus 229E	HCV 229E
Man	Human coronavirus OC43	HCV OC43
Cat	Feline infectious peritonitis virus	FIPV
Mouse	Murine hepatitis virus	MHV
Pig	Porcine epidemic diarrhea virus	PEDV
Pig	Porcine hemagglutinating encephalomyelitis virus	HEV
Pig	Porcine transmissible gastroenteritis virus	TGEV
Turkey	Turkey coronavirus	TCV
Torovirus		
Horse	Berne virus	BEV
Cattle	Breda virus	BRV

A. Serological Relationships

A variety of serological assays have been used to determine the antigenic relationships among coronaviruses. They have included neutralization, hemagglutination-inhibition, immunofluorescence, immunoblotting, enzyme-linked immunosorbent assay (ELISA), and radioimmunoprecipitation using polyvalent and monovalent antisera and monoclonal antibodies (Spaan *et al.*, 1990; Dea *et al.*, 1990). The results of these studies can be summarized by a taxonomy placing the 11 coronavirus species in three antigenic groups. These groups are shown in Table II.

The inclusion of human coronavirus (HCV) 229E in antigenic group 1 has been questioned, essentially because no cross-reactivity could be demonstrated between HCV 229E and a large panel of TGEV-specific monoclonal antibodies (Sanchez *et al.*, 1990). However, the sequence relationships of coronavirus proteins appear to support the inclusion of HCV 229E in this group.

TABLE II. Antigenic Relationships of Coronaviruses

Group 1	Group 2	Group 3
HCV 229E	HCV OC43	IBV
TGEV	MHV	
PEDV	BCV	
CCV	HEV	
FIPV	TCV	

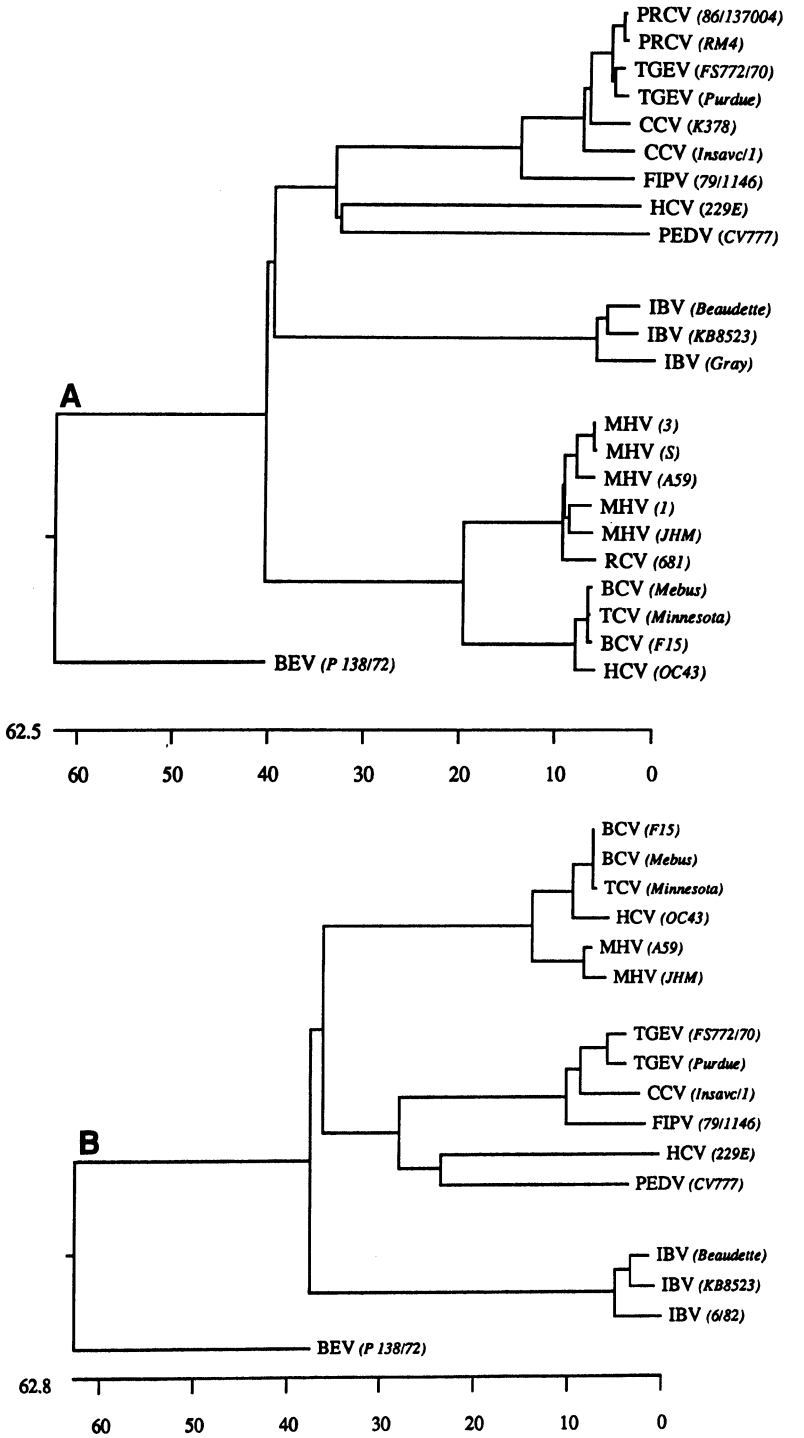
A
PRCV (86/137004)
PRCV (RM4)
TGEV (FS772/70)
TGEV (Purdue)
CCV (K378)
CCV (Insavc/1)
FIPV (79/1146)
HCV (229E)
PEDV (CV777)
IBV (Beaudette)
IBV (KB8523)
IBV (Gray)
MHV (3)
MHV (S)
MHV (A59)
MHV (1)
MHV (JHM)
RCV (681)
BCV (Mebus)
TCV (Minnesota)
BCV (F15)
HCV (OC43)
BEV (P 138/72)
62.5
60 50 40 30 20 10 0
B
BCV (F15)
BCV (Mebus)
TCV (Minnesota)
HCV (OC43)
MHV (A59)
MHV (JHM)
TGEV (FS772/70)
TGEV (Purdue)
CCV (Insavc/1)
FIPV (79/1146)
HCV (229E)
PEDV (CV777)
IBV (Beaudette)
IBV (KB8523)
IBV (6/82)
BEV (P 138/72)
62.8
60 50 40 30 20 10 0

FIGURE 1.

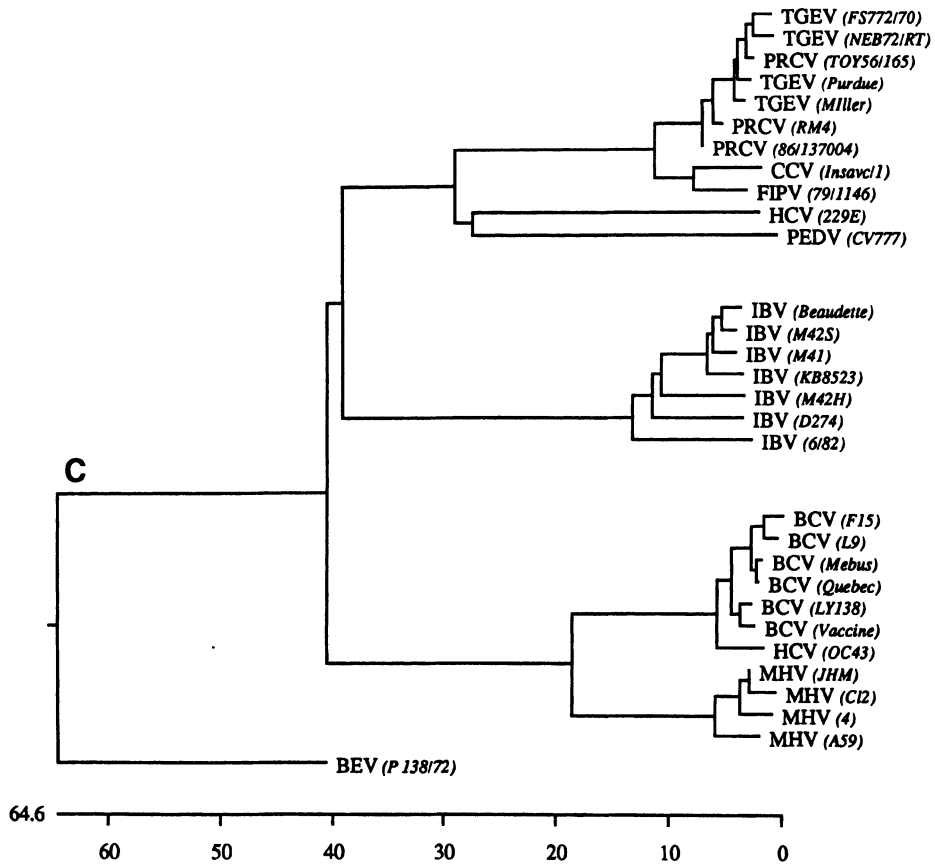

FIGURE 1. Phylogenetic relationships of the (A) nucleocapsid protein, (B) integral membrane protein, and (C) large-surface glycoprotein of coronaviruses. Amino acid sequences were aligned using the "clustal" method (Higgins and Sharp, 1989) and phylogenetic trees were constructed using the neighborhood-joining method (Saitou and Nei, 1987). The analyses were done using the MegAlign module of the Lasergene software suite (DNASTAR Ltd). The phylogenies are rooted assuming a biological clock.

B. Sequence Relationships

The sequence analysis of a number of coronavirus genes, including the complete genomes of avian infectious bronchitis virus (IBV), murine hepatitis virus (MHV), and HCV 229E, provides a database for the analysis of possible evolutionary relationships between coronaviruses. This type of analysis is shown for the coronavirus large-surface glycoprotein, the integral membrane protein, and the nucleocapsid protein in Fig. 1. By and large, the nucleotide sequence data provide a striking confirmation of the conclusions derived from serological analysis. A closely related genetic cluster composed of HCV OC43, MHV, bovine coronavirus (BCV), and turkey coronavirus (TCV) is evident in the cladograms derived for all three proteins. The viruses comprising antigenic

group 1 also form a genetic cluster. However, these viruses are clearly less closely related, and an evolutionary divergence of, in particular, the human coronavirus HCV 229E and the porcine coronavirus porcine epidemic diarrhea virus (PEDV) from the remainder of the group is suggested by the data. It should be remembered that the analyses presented here do not account for the possible role of recombination in the evolution of coronavirus genes (see Chapters 2 and 12).

As evidenced by immunofluorescence, seroneutralization, ELISA, and radioimmunoprecipitation, Berne virus and Breda virus are antigenically related to each other but there is no cross-reactivity with antisera specific for other animal viruses, including coronaviruses. The available data suggest that the cross-reactive torovirus antigen is predominantly the surface glycoprotein (Weiss and Horzinek, 1987). The evidence for antigenic cross-reactivity with human toroviruses is based mainly on immunoelectron microscopy (Beards *et al.*, 1986). At the present time, there is insufficient sequence data to evaluate the phylogeny of toroviruses. Limited but convincing sequence similarities in some of the gene products of toroviruses and coronaviruses (see Chapter 11) support the inclusion of these two genera in one family.

III. GENOME ORGANIZATION

At the present time, the available data suggest that the genomes of coronaviruses and toroviruses contain between 6 and 11 functional ORFs (Spaan *et al.*, 1988) (Fig. 2). The easiest to define are those encoding the structural proteins, in particular, the large surface glycoprotein (S), the integral membrane protein (M), and the nucleocapsid protein (N), which have a specific size and/or location toward the 3′ end of the genome (for an explanation of nomenclature, see Table III). All coronaviruses contain a fourth structural protein ORF encoding the sM protein that is invariably located 5′ proximal to the M protein ORF. Additionally, the genomes of BEV and coronaviruses of the antigenic group 2 (e.g., HCV OC43, MHV, BCV) contain a fifth structural protein ORF, the hemagglutinin-esterase (HE) gene. The location of this ORF in coronaviruses is 5′ proximal to the S protein gene, and in BEV it lies between the M and N protein genes.

The HE gene of coronaviruses and BEV appears to encode a protein with an accessory function that is not required for replication in cultured cells. Thus, in some coronavirus isolates (e.g., MHV A59) and the single BEV isolate, mutation, possibly in cell culture, has resulted in the loss of a functional HE ORF and conversion of these sequences to pseudogenes (Yokomori *et al.*, 1991; Snijder and Horzinek, 1993).

In relation to the ORFs that encode nonstructural proteins, the coronavirus and torovirus genome is dominated by the RNA polymerase locus which consists of two large ORFs encompassing approximately 20,000 nucleotides toward the 5′ end of the genome. The remainder of the coronavirus nonstructural protein ORFs present a diverse and complex pattern both in their number, size, and arrangement. The reasons for the complexity are probably twofold. First, as

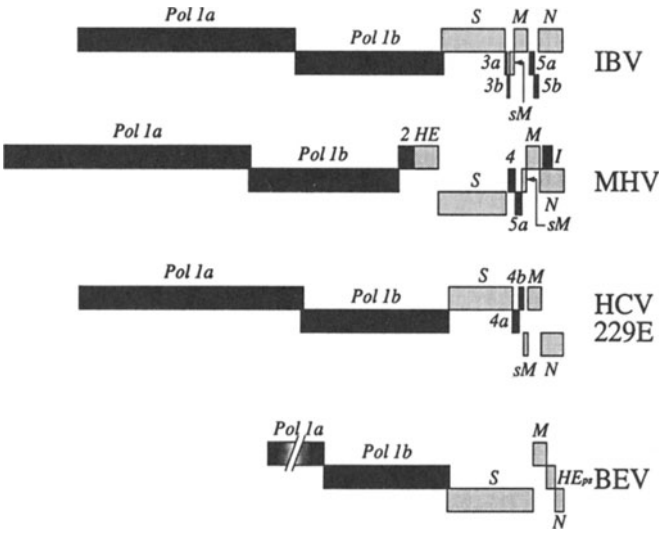

FIGURE 2. The genomic organization of a torovirus (BEV), a group 1 coronavirus (HCV 229E), a group 2 coronavirus (MHV), and a group 3 coronavirus (IBV). The genomic ORFs encoding structural proteins are lightly shaded; those encoding nonstructural proteins are filled. The ORFs are drawn to scale in the correct relative reading frames. ORF Pol 1a is defined as reading frame zero.

mentioned above, many coronavirus genes appear to encode proteins with accessory functions. Thus, under certain conditions (e.g., adaptation and propagation in cultured cells), mutations accumulate that lead to the inactivation and even deletion of these genes. Second, not only divergence from a common ancestor but also RNA recombination appear to be a major driving force in the evolution of coronavirus and torovirus genomes (see Chapter 12). This appears to have introduced a plasticity to the corona/torovirus genome that is exceptional, even among RNA viruses.

The genome maps shown in Fig. 2 should, therefore, be taken as an over-

TABLE III. *Coronaviridae* Structural Proteins[a]

Gene product	Coronavirus		Torovirus	
Nucleocapsid protein	**N**	**Nucleocapsid**	**N**	**Nucleocapsid**
Large-surface glycoprotein	**S**	**Surface**	**S**	**Surface**
	P	Peplomer	P	Peplomer
	E2	Envelope 2		
Integral membrane protein	**M**	**Membrane**	**M**	**Membrane**
	E1	Envelope 1	E	Envelope
Small-membrane protein	**sM**	**Small membrane**		—
Hemagglutinin-esterase glycoprotein	**HE**	**Hemagglutinin-esterase**	**HEps**	**Hemagglutinin-esterase (pseudogene)**
	E3	Envelope 3	ORF 4 product	

[a]The nomenclature shown in bold type is recommended.

view, and isolates that display differences occur. The reader is also cautioned that the nomenclature of the ORFs encoding the nonstructural proteins of coronaviruses is not logical. In some cases, for example, the ORF 3a of canine coronavirus (CCV) and TGEV, the gene products are homologous, while others carrying the same designation, for example, the ORF 3a of IBV, are not.

IV. MORPHOLOGY AND PHYSICOCHEMICAL PROPERTIES

Coronaviruses are described as pleiomorphic but roughly spherical enveloped particles, approximately 60 to 200 nm in diameter with a characteristic "fringe" of 20-nm-long surface projections. An "inner fringe" of short surface projections is sometimes seen on MHV, BCV, TCV, and HCV OC43 particles. Toroviruses are also pleiomorphic enveloped particles, although only 120 to 140 nm in diameter. Toroviruses are disk-, kidney-, or rod-shaped and are likewise decorated with 20-nm surface projections. The nucleocapsid of both coronaviruses and toroviruses has a helical symmetry, but differs remarkably in its morphology. Coronavirus nucleocapsids are extended while those of toroviruses are tubular. These morphological features and the assignment of struc-

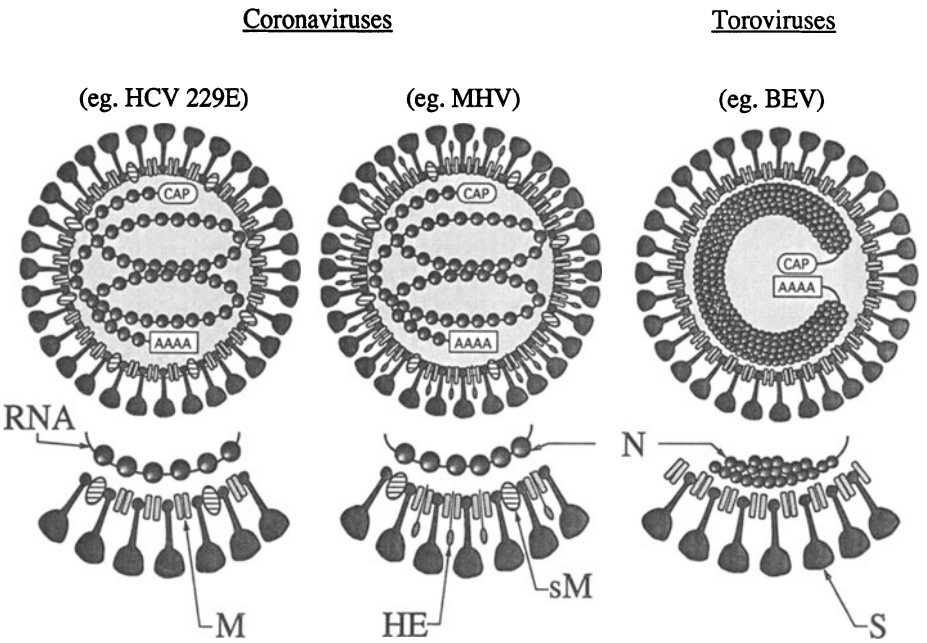

FIGURE 3. The structural components and schematic morphology of torovirus and coronavirus virions. RNA, genomic RNA; cap, a 5′ cap structure; AAAA, a 3′ polyadenylate tract; N, nucleocapsid protein; M, integral membrane protein; S, large-surface glycoprotein; sM, small-membrane protein; HE, hemagglutinin-esterase protein. The stoichiometry of the virion components is shown arbitrarily.

tural proteins to the virus particles are illustrated for a group 1 coronavirus, a group 2 coronavirus, and a torovirus in Fig. 3. In this diagram, the sM protein is depicted as a structural protein of group 2 coronaviruses, although this has not yet been shown experimentally.

Coronaviruses have an estimated molecular mass of 400×10^6 and a buoyant density in sucrose of 1.15 to 1.19 g/cm^3. The buoyant density in CsCl is 1.23 to 1.24 g/cm^3 and the sedimentation coefficient $s_{20,w}$ is 300 to 500. Coronaviruses are sensitive to heat, lipid solvents, nonionic detergents, formaldehyde, and oxidizing agents. Toroviruses have a buoyant density of 1.16 to 1.17 g/cm^3 in sucrose and an estimated sedimentation coefficient $s_{20,w}$ of 400 to 500. Virus infectivity is stable between pH 2.5 and 9.7 but rapidly inactivated by heat, organic solvents, and radiation. There is no inactivation of infectivity by phospholipase C or sodium deoxycholate.

In the last 15 to 20 years many articles that describe the structural, functional, and antigenic properties of the coronavirus and torovirus proteins have been published. However, it was only in 1990 that a standard nomenclature for the structural proteins of coronaviruses was introduced (Cavanagh *et al.*, 1990). This nomenclature has been adopted throughout this book but, to help the reader in the literature, a list of synonyms is presented in Table III. Unfortunately, there is, as yet, no standard nomenclature for the nonstructural proteins of coronaviruses or toroviruses. This is partly because there is insufficient information on the expression, processing, and function of these gene products, but also because of the aforementioned variability in the genomes of corona- and toroviruses. In this respect, the reader is advised to consider each virus species individually.

V. CONCLUSION

The readers of this book will, I hope, appreciate that considerable progress has been made in elucidating many aspects of coronavirus and torovirus biology in a relatively short time. However, there remain many unanswered questions, particularly in areas such as the genesis of corona- and torovirus subgenomic mRNAs, the structure and function of the viral nonstructural proteins, and the pathogenesis of natural infections. I hope that reading this book may stimulate young scientists to address some of these questions.

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