PREFACE

This book summarizes the keynote and plenary speeches and posters of the "Xth International *Nidovirus* Symposium: Toward Control of SARS and Other *Nidovirus* Diseases" that was held in Colorado Springs, Colorado, June 25–30, 2005. The nine previous meetings of scientists investigating the molecular biology and pathogenesis of *coronaviruses*, *toroviruses*, *arteriviruses*, and *okaviruses* were generally held every 3 years since the first meeting was convened in Wurzburg, Germany, in October, 1980. The Xth International Symposium was held just 2 years after the IXth International Symposium (*Nido2003*) in The Netherlands, because of the tremendously increased research on *nidoviruses* that resulted from the discovery that the global epidemic of severe acute respiratory syndrome (SARS) in 2002–2003 was caused by a newly discovered *coronavirus* called SARS-CoV. A record 225 scientists from 14 countries attended the Xth International *Nidovirus* Symposium, and important advances in every aspect of *nidovirus* molecular biology and pathogenesis were reported and discussed. The meeting was divided into 12 sessions, with keynote speakers providing a general review of research pertinent to each one. This volume is a collection of scientific papers presented at the symposium.

Once a *coronavirus* was recognized as the etiological agent of SARS, intensive work by many investigators resulted in determination of the sequence of the virus, engineering of reverse genetics systems, and identification of the host cell receptor used by the virus. With the increased interest in *coronaviruses*, new members of the family associated with human disease were identified. Most notably, HCoV-NL63 and HCoV-HKU1 were recently recognized as important agents of human upper and lower respiratory tract disease. With the identification of new members of the *nidovirus* family, it became important to determine the relationship between these newly recognized viruses and previously classified *nidoviruses*. The *nidovirus* group of the International Committee for Taxonomy of Viruses proposed a taxonomic tree of the *nidoviruses* that is reproduced here (Figure 1). The structure of the viral nucleocapsid, number of subgenomic RNAs and length of the plus strand RNA genomes are strikingly different for each of the *nidoviruses*, although their replication strategies are very similar. This information, coupled with sequencing data, is used to place the newly identified viruses into the pre existing data set. As examples, HCoV-NL63 has been classified as a group 1b *coronavirus*, while SARS-CoV is tentatively classified as a distant member of the group 2 family (group 2b). Other newly identified *nidoviruses*, including those infecting bats, have been similarly analyzed and classified.

The first sessions of the meeting covered "Viral RNA Synthesis." Given the large size of the *nidovirus* replicase gene (gene 1ab, more than 20,000 *nucleotides* for *coronaviruses*)

NEW TAXONOMY

Nidovirales

Coronaviridae		***Arteriviridae***	***Roniviridae***
Coronavirus	*Torovirus*		*Okovirus*
1a HCoV-229E	Berne virus	PRRS	Yellow head virus
1b HCoV-NL63	Breda virus	EAV	Gill associated virus
2a HCoV-OC43			
2b SARS-CoV			
3 IBV			
Helical	*Toroid*	*Icosahedral*	*Rod*

Figure 1. Proposed taxonomy. HCoV-human coronavirus; SARS-CoV, severe acute respiratory syndrome–coronavirus; IBV, infectious bronchitis virus; PRRS, porcine respiratory and reproductive virus; EAV, equine arteritis virus.

and the observation that the gene product is co-translationally cleaved into many proteins, it has been a challenge to determine the functions of individual cleavage products in virus replication. *In silico* analyses suggested roles for these proteins, and advances in genetic manipulations of the viruses coupled with confocal analyses and X-ray crystallography have provided insight into their structures and functions. Certain functional domains of the replicase polyprotein are expressed only by some *coronaviruses*, whereas other domains, such as a uridylate-specific endoribonuclease (NendoU), are encoded in both *coronaviruses* and *arteriviruses*. While the exact functions of these proteins in nidovirus replication need to be determined, much progress has been made in delineating structural domains in some of them and solving their structure.

Much work in the recent past has focused on the structure and function of *nidovirus* structural and nonstructural proteins, encoded downstream of the replicase gene. Each *nidovirus* has an apparently unique set of genes encoding nonstructural proteins that are interspersed with structural genes at the 3' end of the genome. Nothing is known about where the genes encoding these proteins came from and how they were inserted into *coronavirus* genomes. These non structural proteins are apparently not required for virus production *in vitro*, but several contribute to diseases in the infected host. The structures and functions of structural and nonstructural proteins were the topic of "Protein Synthesis, Structure and Processing."

All *nidoviruses* bud intracellularly ("Viral Assembly and Release"). An active area of investigation is to determine the viral and host factors important for virus egress from the cell. Curiously, although the E protein, which has ion channel activity, is believed to be the nidus for virus particle formation, E is not essential for assembly of all *coronaviruses*, because virus-like particles form in its absence. The structures of several *nidoviruses* are being elucidated, which will facilitate understanding of the functions of individual proteins in virion formation.

Nidoviruses use a variety of host cell receptors to enter infected cells ("Viral Entry"), via binding to the virus surface (S) glycoprotein and/or the hemagglutinin esterase glycoproteins found on *coronaviruses* and *toroviruses*. Most group 1 *coronaviruses* enter via interactions with aminopeptidase. An exception is HCoV-NL63, which uses angiotensin converting enzyme 2 (ACE2) as its host cell receptor. SARS-CoV also uses ACE2 to enter cells. Regions on ACE2 important for SARS-CoV entry have been delineated, and the role of host lectins, such as CD209L, in facilitating *coronavirus* entry has also been established. The crystal structure of the receptor binding domain of the SARS S protein bound to ACE2 has also been solved; this structure will be useful not only for understanding virus entry but also for design of antiviral therapies. Elegant studies have also delineated amino acid substitutions in the SARS-CoV S protein that were selected during the 2002–2003 epidemic and facilitated binding to human ACE2, permitting human-to-human spread of the virus and increasing virulence in humans. It is also clear that cleavage of the S protein is critical for fusion of viral and host cell membranes. In many *nidoviruses*, the S protein is cleaved during exit, often by furin or a related serine protease, whereas in others, including SARS-CoV, cleavage occurs during entry and is mediated by cathepsins in endosomes.

Understanding the mechanisms by which nidoviruses cause disease in the infected animal was a major focus of the symposium. Several non-human coronaviruses, including mouse hepatitis virus (MHV) and feline peritonitis virus (FIPV), have been intensively studied for years and are known to cause disease that is partly due to immunopathology. The pathogenesis of these infections was discussed in "Pathogenesis of Non-Human *Coronaviruses*." All strains of MHV uses CEACAM1 to enter cells, but different strains exhibit differences in tissue tropism. For example, MHV-1, a strain that has not been intensively studied in the past, preferentially infects the lower respiratory tract and may serve as a useful model for SARS. *Nidoviruses* also modulate the expression of host cell RNA and protein, presumably to enhance their ability to replicate in infected cells. Induction of immunomodulatory molecules, such as induction of a novel prothrombinase by MHV-3 infection, may also result in severe disease in the infected host.

Arteriviruses cause important diseases, such as equine arteritis and porcine reproductive and respiratory syndrome, in animals. Studies of their replication and pathogenesis have been facilitated by the development of infectious cDNA clones, as described in "Pathogenesis of *Arteriviruses* and *Toroviruses*." These studies will lead to development of vaccines and therapeutics for these important veterinary pathogens.

Prior to the isolation of SARS-CoV as the etiological agent of SARS, human coronaviruses (HCoV-OC43 and HCoV-229E) were known to cause respiratory tract infections, and occasionally to be associated with outbreaks of diarrhea. The identification of SARS-CoV, HCoV-NL63, and HCoV-HKU1 increased the interest in pathogenesis of human *coronavirus* infections ("Pathogenesis of Human *Coronaviruses*"). Several animals can be infected with SARS-CoV, but none of them reproducibly develops the pulmonary disease observed in infected humans. SARS-CoV–infected ferrets are considered the most promising of the available animal models for SARS. Other approaches include infection of human airway cells with SARS-CoV or with retroviruses pseudotyped with the SARS-CoV S protein, because these cells are primary targets for the virus in infected humans. Another approach for delineating the functions of some SARS-CoV nonstructural proteins is to develop chimeric *coronaviruses* of lab animals that express individual SARS-CoV proteins. The interest in

SARS-CoV and HCoV-NL63 has spilled over into research into the pathogenesis of HCoV-229E and HCoV-OC43. These two viruses show striking differences in their ability to cross species. HCoV-229E infects only humans, and infection of mice transgenic for the virus receptor (human *aminopeptidase*) is not robust. In contrast, HCoV-OC43 readily adapts to infect other species and in mice causes a profound infection of neurons after only a few *in vivo* passages.

The papers comprising the final section, "Vaccines, Antiviral Drugs, and Diagnostics," reflect the importance of SARS-CoV as a human pathogen. Efforts to develop inactivated, subunit vaccines and live attenuated vaccines are underway. Testing of these vaccines will benefit from the development of an animal model for SARS. Passive immunization with anti-SARS-CoV antibody may also be used during an epidemic, and human monoclonal antibodies that neutralize the virus have been developed. Crystal structures of proteins, such as the SARS-CoV main protease, will also lead to development of drugs that inhibit SARS-CoV replication with minimal effect on host cell functions. Finally, antisense RNA and siRNA methodologies are being developed as novel approaches to SARS therapy.

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