Molecular Biology of the SARS-Coronavirus

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Editor

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Cover Illustration: 3D model of SARS-CoV, with a wedge cut out of it to reveal the nucleocapsid (see Chap. 3 by Daniel R. Beniac and Timothy F. Booth)

Background: HL-CZ cells transfected with SARS-CoV Spike construct and incubated with anti-Spike human monoclonal antibody followed by secondary FITC-labeled anti-human antibody (see Chap. 18 by T. Narasaraju, P.L. Soong, J. ter Meulen, J. Goudsmit and Vincent T.K. Chow)

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Foreword

SARS was the first new plague of the twenty-first century. Within months, it spread worldwide from its "birthplace" in Guangdong Province, China, affecting over 8,000 people in 25 countries and territories across five continents. SARS exposed the vulnerability of our modern globalised world to the spread of a new emerging infection. SARS (or a similar new emerging disease) could neither have spread so rapidly nor had such a great global impact even 50 years ago, and arguably, it was itself a product of our global inter-connectedness. Increasing affluence and a demand for wild-game as exotic food led to the development of large trade of live animal and game animal markets where many species of wild and domestic animals were co-housed, providing the ideal opportunities for inter-species transmission of viruses and other microbes. Once such a virus jumped species and attacked humans, the increased human mobility allowed the virus the opportunity for rapid spread. An infected patient from Guangdong who stayed for one day at a hotel in Hong Kong led to the transmission of the disease to 16 other guests who travelled on to seed outbreaks of the disease in Toronto, Singapore, and Vietnam, as well as within Hong Kong itself. The virus exploited the practices used in modern intensive care of patients with severe respiratory disease and the weakness in infection control practices within our health care systems to cause outbreaks within hospitals, further amplifying the spread of the disease. Health-care itself has become a two-edged sword.

While SARS exposed the vulnerabilities of the modern human condition, it also highlighted the global capacity for a rapid public health and scientific response to an emerging infectious disease threat. Public health and scientific responses succeeded in identifying the causative agent, developing diagnostic tests, and interrupting the spread of the outbreak. The complete virus genome was fully deciphered within weeks and in the ensuing months and years saw an outpouring of scientific research about the disease and its causative agent, the SARS coronavirus. The natural animal reservoir (bats) and amplifier hosts were defined, the virus receptor on human cells identified and novel antiviral drugs and candidate vaccines developed. This resurgence of attention on coronaviruses led to a much better scientific understanding

about the biology of the coronaviruses in general, the discovery of two new coronaviruses that cause human disease (NL-63 and HKU-1) and a range of novel coronaviruses that infect animals.

The precursor of the SARS coronavirus still persists in its natural reservoir host and whether this precursor virus will readapt to humans at some time in the future remains unknown. However, the human adapted SARS coronavirus remains in laboratories and may yet escape, either inadvertently or through malicious action. We thus need to remain vigilant to the re-emergence of a SARS-like disease. What is certain, however, is that we will be confronted with other emerging infectious diseases in the decade ahead and that most of these diseases will arise from an animal reservoir. Thus, the mechanisms of the emergence of SARS serve as an excellent case-study to better understand how viruses jump species-barriers to cause disease outbreaks in humans. The synthetic reconstruction of an infectious bat-SARS-like precursor virus, the largest life form to be created by synthetic biology to date, has provided an excellent model for understanding such mechanisms. This book, which includes the current understanding of the molecular biology of SARS coronavirus and its applications to understanding pathogenesis, host responses, inter-species transmission, therapeutics and vaccine design, is therefore timely.

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Preface

The SARS outbreak took the whole world by surprise in November 2002. It was the most unprecedented epidemic outbreak in recorded history and the first major new infectious disease of this century, unusual in its high morbidity and mortality rates and in strategically taking advantage of modern international travel to propagate itself around the world. What followed was a global havoc created by this disease, bringing the healthcare system of affected areas to a grinding halt, affecting healthcare providers, disrupting scheduled emergency surgeries and vital treatment to patients with serious conditions, overloading hospitals with infected cases, forcing public events to be cancelled, and schools, and borders to be closed. The economic impact on individuals and businesses was profound, downregulating tourism, education, and employment.

The epidemic was completely different from all known traditional atypical types of pneumonia because patients experienced lack of oxygen at the onset of the disease and hence required the aid of modern respiratory equipment to breathe. This syndrome was contagious enough to infect a substantial number of people widely and easily. In our days of medical advancement and high technology, which has subsequently led to increased life spans and longevity, a growing confidence had emerged in mankind that it had now achieved the ability to overcome the most complicated life-threatening situations. SARS shattered this confidence and made us realize once again that there are hundreds of dangerous and virulent microorganisms living on the other side of the border that can kill humans. What separates us from them is only the species barrier.

This is not the first time the species barrier has been crossed. The SARS outbreak was just another outbreak in South-East Asia, the breeding ground for notorious viruses. The current novel H1N1 swine-flu outbreak that emerged from Mexico, bird-flu H5N1 influenza in Hong Kong in 1996, human enterovirus 71 in Malaysia, Taiwan, and Singapore in 1977, 1998, and 2000 respectively, and the Nipah virus in Malaysia and Singapore in 1998, are all similar examples. The SARS outbreak was a short-lived near-pandemic situation that originated in the Guangdong province of south China in late 2002 and was efficiently contained by July 2003, with 8,096

known infected cases and 774 deaths (a case-fatality rate of 9.6%) infecting individuals from 37 countries worldwide (mortality by age group: below 1% for people aged 24 or younger, 6% for those aged 25–44, 15% for those aged 45–64 and more than 50% for those over 65). If SARS had not been fully contained, the world would have faced a full-blown pandemic. We must not forget that SARS has not been eradicated (e.g., smallpox). It is still present in its natural host reservoirs and carries the threat and potential to return into the human population any time.

We were able to subvert a potentially explosive spread of the new coronavirus (SARS-CoV) outbreak thanks to WHO's global alert, getting together an emergency network of 11 leading laboratories from 9 countries to investigate this new virus. Within a short span of 1 month, these laboratories did a commendable job by tracing the viral etiology and developing a diagnostic test. Over the years, much has been learnt about this new SARS-CoV; however, our knowledge on the molecular biology of SARS-CoV, its life-cycle, infection, and pathogenesis still remain unclear. This virus is mysterious in its ways and this book looks at various molecular aspects of this virus which help us in understanding these complexities.

Prior to the SARS outbreak, human coronaviruses were only associated with mild diseases. SARS-related CoV became the first coronavirus to cause severe disease in humans. In April 2003, the complete genome sequence of the SARS-CoV was revealed. The genome contains unique 5' and 3' UTRs (untranslated regions) containing higher-order structures which play essential roles in viral transcription and replication, assisted by cellular proteins to perform RNA synthesis, a model elegantly reviewed by Liu and Leibowitz in this book. The SARS-CoV genome contains five major open reading frames (ORFs) that encode the replicase polyprotein, the spike (S), envelope (E), and membrane (M) glycoproteins, and the nucleocapsid protein (N). S binds species-specific host cell receptors and triggers a fusion between the viral envelope and the cell membrane. Lambert's chapter clearly describes the basic cell biology of ACE2 and Pöhlmann's chapter elaborates on the S-ACE2 interface. Receptor binding and the subsequent structural changes that result have been described in detail by Beniac and Booth. The S protein is the virulence factor in many different coronaviruses and the principal viral antigen that elicits neutralizing antibody on behalf of the host. To study this, Chow's lab has undertaken whole transcriptome analysis of S transfected host cells and identified novel pathways that become altered. Replicase proteins have been extensively discussed in the chapters by Ziebuhr and Canard. Immediate early proteins, like the RNA dependent RNA polymerase (RDRP) and proteases, are responsible for preparing the infected cell for virus takeover. Dinman describes programmed -1 ribosomal frameshifting as an essential and unique feature of the virus for the translation of these proteins. The overlapping polyproteins 1a and 1ab are extensively cleaved by the internally encoded SARS-CoV proteases, Mpro, and PLpro and are extensively discussed by Chang in his chapter. The N protein forms the capsid and also plays several regulatory roles during viral pathogenesis which have been described by Surjit and myself. Cell type specific apoptosis induction of host cells by viral proteins has been elegantly described by Hermann Schätzl et al. Three chapters are dedicated to describe the current knowledge on accessory proteins by

Pekosz, Sun, and Tan. Sheahan and Baric's chapter and Li and Xu's chapter describe exhaustively the pathogenesis and protective immunity against SARS-CoV in humans. Cell signaling and associated lung fibrosis due to TGF-/Smad pathways are discussed in the chapters by Mizutani and Chen, respectively. The importance and application of retroviral pseudotypes for highly pathogenic diseases like SARS, using surrogates of the live virus for neutralization assays, has been described by Nigel Temperton.

I wish to congratulate and thank all the contributing authors for the exhaustive coverage of their respective subjects and publication of this book. We hope the readers find this book a consolidated compilation of our current understanding of the molecular biology of SARS-CoV.

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Contents

Part I
Viral Entry

Chapter 1
Cellular Entry of the SARS Coronavirus: Implications for Transmission, Pathogenicity and Antiviral Strategies

Ilona Glowacka, Stephanie Bertram, and Stefan Pöhlmann

Abstract A novel coronavirus was identified as the causative agent of the lung disease severe acute respiratory syndrome (SARS). The outbreak of SARS in 2002/2003 was associated with high morbidity and mortality and sparked international research efforts to develop antiviral strategies. Many of these efforts focussed on the viral surface protein spike (S), which facilitates the first indispensable step in the viral replication cycle, infectious entry into target cells. For infectious cellular entry to occur, the S protein must engage a cellular receptor, the carboxypeptidase angiotensin-converting enzyme 2 (ACE2). The interface between ACE2 and S protein, which has been characterized at the structural level, constitutes a key target for vaccines and inhibitors, and is believed to be an important determinant of viral pathogenesis and interspecies transmission. In this chapter, we will discuss how SARS-S mediates cellular entry and we will review the implications of this process for SARS coronavirus (SARS-CoV) transmission, disease development and antiviral intervention.

1.1 Introduction

The emergence of the severe acute respiratory syndrome coronavirus (SARS-CoV) in Guangdong Province, China, in 2002, and its subsequent spread in Asia and Canada clearly exemplified the vulnerability of societies and economies to a novel, highly pathogenic respiratory agent (Stadler et al. 2003; Peiris et al. 2003b). The outbreak, which was halted solely by the quarantine of exposed individuals and the use of conventional prevention measures such as surgical masks, was paralleled by an international, collaborative scientific effort to develop means for therapeutic and

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preventive intervention (Peiris et al. 2004; Stadler and Rappuoli 2005). The basis for the development of successful antiviral strategies is a thorough understanding of the molecular biology underlying viral amplification and pathogenesis, and many significant discoveries have been made in the SARS field since the identification of the virus early in 2003 (Drosten et al. 2003; Ksiazek et al. 2003; Peiris et al. 2003a). Several of these findings provided important insights into the structure and function of the viral spike (S) protein, which is used by the virus as the key to bind and enter host cells (Hofmann and Pöhlmann 2004). The most well-known examples are the identification of angiotensin-converting enzyme 2 (ACE2) as the host factor which is engaged by the viral S protein for infectious entry into cells, and the elucidation of the structure of the S protein receptor binding domain (RBD) in complex with ACE2 (Li et al. 2003, 2005a). These findings have major implications not only for vaccine and inhibitor development but also for our understanding of the SARS zoonosis, since adaptation of SARS-S to robust usage of human ACE2 was probably of key importance for efficient SARS-CoV spread in humans (Li et al. 2005a, 2005c). In this chapter, we will discuss how SARS-CoV gains access to target cells and how this process can be inhibited. In addition, we will review how the molecular interactions underlying SARS-CoV entry impact viral pathogenesis and interspecies transmission.

1.2 The Spike Protein: Key to the Host Cell

The SARS-Sprotein is a type I transmembrane protein, which comprises 1,255 amino acids and contains 23 consensus signals for *N*-linked glycosylation (Hofmann and Pöhlmann 2004). S protein is synthesized in the secretory pathway of infected cells. It contains an N-terminal signal sequence, which mediates import of the nascent protein into the endoplasmatic reticulum, where the protein is folded and modified with mannose-rich carbohydrates. Upon transport of the protein into the Golgi apparatus, most, if not all, of the high-mannose carbohydrates are processed into complex glycans (Nal et al. 2005). Evidence of *O*-glycosylation of SARS-S has not been reported. A novel dibasic ER retrieval motif in the cytoplasmic tail of SARS-S promotes accumulation of the S protein at the ER–Golgi intermediate compartment and the Golgi region (McBride et al. 2007), the sites where progeny particles are assembled (Stertz et al. 2007; Siu et al. 2008). Formation and budding of new particles are driven by the membrane protein (M), the envelope protein (E) and the nucleocapsid protein (N) (Huang et al. 2004; Hsieh et al. 2005; Siu et al. 2008); interactions with the M protein might facilitate S protein incorporation into particles. Trimers of the S protein protrude from the viral envelope and provide virions with a crown (Lat. *corona*) -like appearance, from which the name "coronaviruses" is derived.

The domain organization of SARS-S resembles that of several well-characterized viral membrane proteins, such as influenza virus hemagglutinin (HA) and human immunodeficiency virus (HIV) envelope protein (Env) (Hofmann and Pöhlmann 2004).

These proteins employ comparable strategies to facilitate fusion of viral and host cell membranes and are termed class I fusion proteins (Kielian and Rey 2006). They are distinguished from class II fusion proteins (Kielian 2006), found, for example, on flavi- and alphaviruses, by their distinct spatial organization and the particular configuration of the functional elements required for fusion with target cells: class I fusion proteins are inserted perpendicular to the viral membrane and contain an N-terminal surface unit (SU) and a C-terminal transmembrane unit (TM). The globular SU interacts with cellular receptors, while the TM promotes

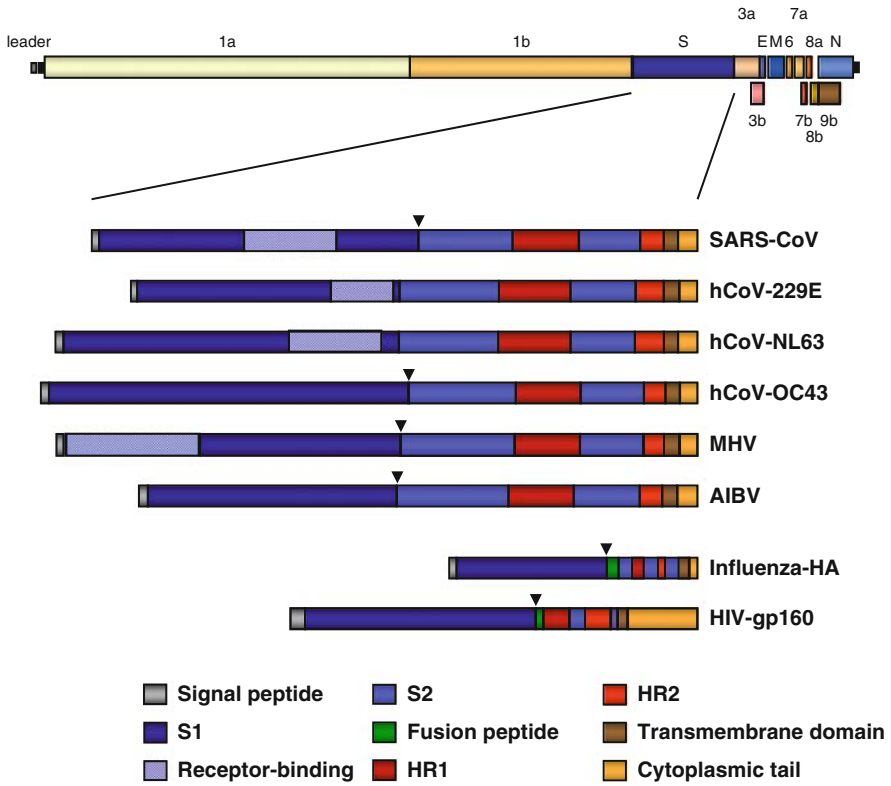

Fig. 1.1 Domain organization of coronavirus S proteins (adapted from Hofmann and Pöhlmann 2004). The position of the S protein open reading frame in the SARS-CoV genome is indicated in the *upper panel*. Coronavirus S proteins exhibit a domain organization characteristic for class I fusion proteins. The domain organization of prototype class I fusion proteins, the HIV envelope protein, and the influenza virus HA is shown below. A signal peptide is located at the N terminus and mediates import of the nascent protein into the secretory pathway of infected cells. The surface unit S1 contains a receptor binding domain (RBD), which allows engagement of cellular receptors for infectious entry. The transmembrane unit (S2) harbors functional elements pivotal to membrane fusion: a fusion peptide, two helical regions, and a transmembrane domain. Proteolytic cleavage into the S1 and S2 subunits by host-cell proteases is indicated by a *triangular arrow*. AIBV: avian infectious bronchitis virus; hCoV: human CoV; HR: helical region; MHV: murine hepatitis virus; SARS: severe acute respiratory syndrome