Wrapping up : nidovirus membrane structures and innate immunity

Oudshoorn, D.

Chapter 1

General introduction

GENERAL INTRODUCTION

In recent years, the enormous impact virus outbreaks can have on public health has been illustrated by the epidemics of Zika virus in South America, Ebola virus in West Africa, and Middle East respiratory syndrome-coronavirus (MERS-CoV) in the Arabian Peninsula. Infections with such (re-)emerging viruses can have devastating effects for individuals, while at the same time having worldwide societal and economic impact. Virus outbreaks often accelerate research efforts in order to develop rapid and reliable diagnostic methods and ultimately find therapies and vaccines for life-threatening infections. For example, the severe acute respiratory syndrome-coronavirus (SARS-CoV) sparked renewed interest in research on coronaviruses (and by extension nidoviruses). The order *Nidovirales* currently encompasses four virus families, *Coronaviridae*, *Arteriviridae*, *Roniviridae* and *Mesoniviridae* (recently revised, for details see https://talk.ictvonline.org/taxonomy/). For the latter two, only a limited number of species have been discovered and the replication of these viruses remains to be studied in detail (1-3). The arterivirus family currently does not include known human pathogens. Instead, its members are known to infect livestock (equine arteritis virus (EAV) and porcine reproductive and respiratory syndrome virus (PRRSV)), rodents or non-human primates (simian hemorrhagic fever virus (SHFV) and related viruses) (4). EAV, being the prototypic as well as the first described arterivirus (5), has been well-studied (4, 6). During the past 25 years, PRRSV has posed a major threat to the swine industry worldwide (7, 8). Substantial research investments have been made to develop vaccines that may prevent PRRSV outbreaks (4, 9), although these are currently still insufficiently effective to control PRRSV spread. The molecular biology of EAV, the first nidovirus for which a reverse genetics system was developed (10), has been studied in considerable detail and the virus is considered a model for both other arteriviruses (like PRRSV) and the nidovirus order at large, including the distantly related coronaviruses (2). Knowledge gained from studies of EAV replication has been invaluable in enhancing our understanding of the molecular biology of other members of the order *Nidovirales*.

Currently the coronavirus family includes the largest number of classified members among the *Nidovirales* families, which are distributed over the genera *Alpha-*, *Beta-*, *Gamma-* and *Deltacoronavirus* (subfamily *Coronavirinae*) and the subfamily *Torovirinae* (https://talk.ictvonline.org/taxonomy/). In 2003, the previously unknown betacoronavirus causing SARS emerged and was the first coronavirus causing severe human disease with a potentially lethal outcome (11). Since then, no further SARS-CoV outbreaks have occurred, but a decade later another betacoronavirus that can cause severe SARS-like respiratory disease emerged in the Arabian Peninsula. This Middle East respiratory syndrome coronavirus (MERS-CoV) was first isolated from a human case in 2012 (12). The introduction of both SARS-CoV and MERS-CoV into the human population likely were zoonotic events, as close relatives of both viruses circulate in bats and dromedary camels, respectively (13). The two outbreaks have highlighted the potential threat that coronaviruses can pose to public health, which merits further research to dissect their replication and evolution. No specific strategies to control or prevent coronavirus infections are currently available (14), although

the MERS-CoV outbreak has sparked efforts to develop both vaccines and antiviral drugs (15). A better understanding of the fundamental aspects of coronavirus replication will help to design novel strategies to combat these infections.

Like all other +RNA viruses of eukaryotes, a group that includes important human pathogens like poliovirus, hepatitis C virus, dengue virus, and zika virus, nidoviruses replicate in the cytoplasm of the infected cell. After virus entry, the viral genome, which is of mRNA polarity, is released into the cytosol and can be directly translated by host ribosomes. Translation of +RNA virus genomes generally results in large polyproteins that are cleaved by viral and/or host proteases to release functional smaller subunits, such as the enzymes involved in RNA replication an capping. All +RNA viruses of eukaryotes modify intracellular membranes to accommodate their replication machinery (16-19). The viral RNA-synthesizing machinery is associated with these membrane structures, which will thus be referred to as 'replication organelles' (ROs) in this thesis. The viral ROs are thought to constitute a micro-environment that facilitates viral RNA synthesis. Furthermore, they could play a role in the spatial coordination of viral replication in the cell, by contributing to the compartmentalizing of certain steps of the viral cycle. Finally, ROs may aid in shielding replicating viral RNA from detection by the host cell's innate immune system. In the case of the arteriviruses and coronaviruses, the ROs mainly consist of a reticulovesicular network (RVN) of double-membrane vesicles (DMVs) that are interconnected with each other and (modified) ER membranes (20-22). RO formation is generally driven by specific viral proteins and their transient expression can in some cases suffice to induce the formation of very similar membrane structures (23-28). For arteriviruses, it has been shown that expression of two viral replicase subunits (non-structural protein (nsp) 2 and 3) results in the formation of DMVs similar to those observed during arterivirus infection (24). SARS-CoV DMV formation was claimed to require co-expression of three replicase subunits (nsp3, nsp4, and nsp6) (28). The biogenesis of nidovirus replication organelles will be reviewed extensively in **Chapter 2**.

RNA viruses can evolve very quickly, aided by their high mutation rate. This feature provides them with great adaptability, including the potential for host switching and the evasion of both the innate and the adaptive immune responses. While the adaptive immune response can develop towards specific viral epitopes, the innate immune system targets so-called pathogen-associated molecular patterns (PAMPs), which in the case of viruses can include double-stranded RNA, 5' triphosphate-bearing RNA, cytosolic DNA, or viral capsids. PAMPs can be recognized by cellular pathogen recognition receptors (PRRs), which subsequently induce the expression of interferon-stimulated genes (ISGs) in the infected as well as neighboring cells (29-31). The proteins expressed from ISGs restrict the replication of a whole range of viruses, either by targeting viral functions directly or by modulating host processes involved in virus replication, which leads to a so-called "antiviral state". The importance of the innate immune system is evident from the consequences of defects in – for example – the function of STAT1, a critical protein in the interferon-driven response to infection whose inactivation leads to severe susceptibility to viral infections in humans (32), illustrate the importance of the innate immune system. In line with the

everlasting evolutionary battle between pathogens and their hosts, viruses have developed numerous strategies to prevent the production of type I interferon, limit the effect of type I interferon signaling, or interfere with the antiviral effects of ISGs (33). Not surprisingly, ISGs (and other immunity-related genes) are among the fastest evolving genes (34), highlighting the continuing 'arms race' between pathogens and the innate immune system of their host.

THESIS OUTLINE

This thesis discusses the structure and function of the ROs of arteriviruses and coronaviruses, in particular details of their architecture, the viral factors required for their biogenesis, and novel insights regarding innate immune responses that may counter RO formation, possibly also targeting other +RNA viruses. In **Chapter 2** the current literature on +RNA virus ROs is reviewed, with a special focus on the membrane structures induced in cells infected with arteriviruses and coronaviruses, including their biogenesis and the viral proteins involved. **Chapter 3** further explores this topic and includes new data on the biogenesis of arteriviruses DMVs. Using electron tomography, intermediate structures were visualized that provide new clues on the sequence of membrane remodeling events leading to DMV formation. In addition, the effect on DMV formation was assessed when the third transmembrane nsp of EAV (nsp5) was co-expressed with nsp2 and nsp3. **Chapter 4** describes the identification of the viral proteins required for biogenesis of MERS-CoV DMVs. Although previous work on SARS-CoV led to the conclusion that all three transmembrane nsps (nsp3, nsp4 and nsp6) are required for DMV formation, electron microscopy and electron tomography studies now revealed that – for both MERS-CoV and SARS-CoV – co-expression of nsp3 and nsp4 sufficed to achieve DMV formation. Furthermore, these DMVs were organized in a RVN, similar to what has previously been observed in coronavirus-infected cells. This study also emphasized clear parallels between the viral proteins required for the biogenesis of the ROs of arteriviruses and coronaviruses. **Chapter 5** switches gears by reviewing the literature regarding the induction and regulation of the cellular innate immune response against viruses. The chapter is mainly focused on the pathways leading to the production of type I interferons and the role that post-translational modification with ubiquitin and other ubiquitin-like modifiers plays in the regulation of the innate immune response. Whether +RNA virus ROs may be targeted by the innate immune system is then explored in **Chapter 6**, which analyzes the impact of type I interferon treatment on the induction of DMV formation by arterivirus nsp2-3. Interferon-β treatment resulted in a sizeable reduction of the number of DMVs observed. Moreover, double-membrane sheets, a putative intermediate of DMV biogenesis, accumulated following Interferon-β treatment, which suggests that DMV biogenesis was impaired. Finally, **Chapter 7** summarizes the work presented in this thesis and discusses the findings in the broader context of +RNA-viral ROs.