Summary

During the outbreak of the Severe Acute Respiratory Syndrome (SARS) in 2003, about 8000 people fell ill of which about 10% did not survive the infection. For about 4 months, the SARS outbreak had a profound effect on public life and the global economy. It was ultimately contained using quite traditional measures, like respiratory protection and quarantining of patients. Essentially there were no alternatives, since coronaviruses were relatively poorly studied and vaccines or antiviral drugs targeting this new virus were not available.

Positive-stranded RNA viruses, to which the SARS-coronavirus (SARS-CoV) belongs, replicate in the cytoplasm of infected host cells. Their replication complexes are commonly associated with modified host cell membranes. Membrane structures supporting viral RNA synthesis range from distinct spherular membrane invaginations to more elaborate webs of packed membranes and vesicles. Generally, their ultrastructure, morphogenesis, and exact role in viral replication remain to be defined.

Poorly characterized double-membrane vesicles (DMVs) were previously implicated in SARS-CoV RNA synthesis. To obtain more information about the 3-dimensional structure of coronavirus-induced membrane structures, electron tomography of cryofixed infected cells was applied in chapter 2. This analysis defines a unique reticulovesicular network (RVN) of modified endoplasmic reticulum that integrates convoluted membranes, numerous interconnected DMVs (diameter 200–300 nm), and “vesicle packets” apparently arising from DMV fusion. The convoluted membranes were most abundantly immunolabeled for viral replicase subunits. However, double-stranded RNA, presumably revealing the site of viral RNA synthesis, mainly localized to the DMV interior and was enclosed by a double membrane. Since connections between the DMV interior and cytosol could not be discerned, the analysis raises several questions about the mechanism of DMV formation and the actual site of SARS-CoV RNA synthesis. The data described in chapter 2 document the extensive virus-induced reorganization of host cell membranes into a network that is used to organize viral replication and possibly hide replicating RNA from antiviral defense mechanisms.

In chapter 3, it was investigated how the early secretory pathway interacts with the RVN and the viral replication/transcription complex (RTC) that is anchored to it. When the secretory pathway was disrupted by brefeldin A (BFA) treatment shortly after the start of infection, RVN formation and viral RTC activity were not blocked and continued up to 11 h post infection, although RNA synthesis was reduced by about 80%. In vitro RTC assays, using membrane fractions from infected cells, demonstrated that BFA does not directly interfere with the activity of the viral RNA-synthesizing enzymes. Confocal microscopy studies showed that early secretory pathway components are not associated with SARS-CoV-induced replication sites, although our studies revealed that infection induces a remarkable redistribution of the translocon subunit Sec61α. Ultrastructural studies, including electron tomography,
revealed that the formation of the RVN and all its previously documented features can occur in the presence of BFA, despite differences in volume and morphology of the network. We therefore conclude that early secretory pathway proteins do not play a direct role in RVN morphogenesis or functionality of the SARS-CoV RTC. The BFA-induced disruption of ER integrity and functionality probably affects the overall quality of the membrane scaffold that is needed to support the viral RTC and/or the availability of specific host factors, which in turn compromises viral RNA synthesis.

Translation of the viral RNA genome produces the replicase polyproteins that direct RNA synthesis. Although it is generally accepted that viral RNA synthesis initially depends on the same RNA template, it was unknown which effect translation inhibition has on the functionality of the coronavirus RTC and development of the RVN. Therefore, in chapter 4, the effect of translation inhibition on the RNA synthesis of SARS-CoV and mouse hepatitis virus was studied by using cycloheximide and puromycin. Both inhibitors prevented the usual exponential increase in viral RNA synthesis, with immunofluorescence and electron microscopy indicating that RVN development came to a standstill. Nevertheless, limited RNA synthesis was supported, implying that continued translation is not an absolute requirement and suggesting a direct link between RVN formation and accumulation of coronavirus proteins.

Also the replicase proteins of the arteriviruses are associated with unusual double-membrane vesicles (DMVs; diameter ~100 nm), which were previously proposed to derive from the endoplasmic reticulum (ER). Using advanced electron microscopic techniques, including electron tomography and electron spectroscopic imaging, an in-depth ultrastructural analysis of cells infected with the prototypic arterivirus equine arteritis virus (EAV) was performed in chapter 5. It was established that the outer membranes of EAV-induced DMVs are interconnected with each other and the ER, thus forming a reticulovesicular network that – to a certain extent – resembles membrane structures accommodating the RNA synthesis of the very distantly related coronaviruses. A clear and striking parallel between coronavirus and arterivirus DMVs is the accumulation in their interior cavity of double-stranded RNA, the presumed intermediate of viral RNA synthesis. However, openings connecting DMV interior and cytosol were only rarely observed, and likely represented fixation or staining artifacts. Also semi-permeabilization and nuclease digestion experiments suggested that the interior of EAV-induced DMVs is inaccessible from the cytosol, implying that the double-stranded RNA is compartmentalized by membranes. As a novel approach to visualize and quantify the RNA content of viral replication structures, we explored electron spectroscopic imaging of DMVs, which revealed the presence of an RNA amount equaling up to a few dozen copies of the EAV genome. Finally, we visualized a peculiar network of EAV nucleocapsid protein-containing protein sheets and tubules, which appears intertwined with the RVN. This potential intermediate in nucleocapsid formation suggests that arterivirus RNA synthesis and virion assembly are coordinated in intracellular space.
Despite significant morphological differences between the replication networks induced by SARS-CoV and EAV, it seems that RVN formation is a common property of cells infected with nidoviruses. Together with biochemical studies of the viral enzyme complex, our ultra-structural description of this replication network will aid to further dissect the early stages of the nidovirus life cycle and its virus-host interactions.