SARS-CoV2 : Characterization of viral replication centers

The novel coronavirus SARS-CoV2 belongs to a large group of positive strand RNA viruses. After infection of host cells, the virus releases its RNA-genome, which directly serves as matrix for translation of viral proteins (Figure 1a, b). The first translation product is a large polyprotein, which comprises eleven non-structural proteins (nsp1-11). Later, a longer version of the polyprotein is produced that comprises nsp1 to nsp16. Early and late nsp's serve different functions. Nsp1 to nsp10 are mainly involved in suppressing the anti-viral innate immune response of host cells. Nsp3, nsp4 and nsp6 remodel the endoplasmic reticulum to induce the formation of double-membrane vesicles (DMVs), that are serving as viral replication centers (Figure 1c). Nsp12 is a RNA dependent RNA polymerase which is responsible for replication of viral RNA. The other late nsps associates with nsp12 to from a viral replication and transcription complex that colocalizes with DMVs. How DMVs are generated is, however, unknown. In the proposed project, the formation of DMVs will be investigated by applying a combination of biophysical, biochemical and cell biological methods. The presences of specific proteins at DMVs in SARS-CoV2 infected cells will be analyzed by correlative light electron microscopy. Interaction of proteins will be studied by fluorescence lifetime imaging. Another part of the project is to reconstitute the formation of DMVs in non-infected cells by expressing the viral proteins nsp3, nsp4 and nsp6. The interactions of these proteins with proteins of the host cell will be analyzed to reveal how DMVs are formed. The work will take place at the campus of the Institut Pasteur Paris.

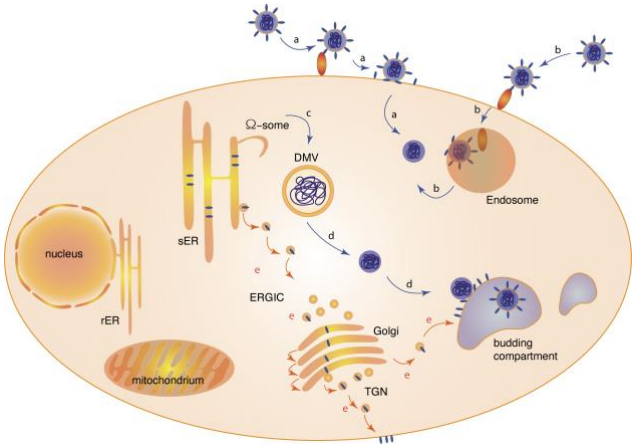

Figure 1. Viral infection cycle.

The infection cycle of coronaviruses is shown as scheme. (a) Viral entry by binding to the cell surface receptor ACE2 (red) and fusion of the spike protein after proteolytic processing at the plasma membrane by TMPRSS2. (b) Viral entry by taking advantage of the endocytic pathway involving clathrin dependent or independent endocytosis and proteolytic processing in lysogenic compartments involving cathepsin L. (c) Formation of double membrane vesicles (DMVs) from omegasomes at the ER. (d) DMVs serve as replication organelles to produce viral genomic RNA. The interaction of exported RNA and viral N-proteins give rise to viral capsids (dark blue) that are delivered to budding compartments (light blue). (e) Trafficking of viral structural proteins (dark blue) from the ER to the Golgi and the plasma membrane following the exocytotic pathway. Proteins need to be diverted to reach viral budding compartments at which capsids bud into the lumen. The compartments fuse with the plasma membrane to release viruses.

Supervisor:

Thomas Wollert, Directeur de Recherche,

Membrane Biochemistry and Transport,

CNRS UMR3691,

Institut Pasteur Paris,

28 rue du Dr Roux, 75015 Paris