SARS-CoV-2 remodels endosomal membranes to generate replication organelles

0234

Yuexuan Chen[1] and Ruth Serra-Moreno[1]

[1]Microbiology and Immunology

University of Rochester, School of Medicine and Dentistry, Rochester, NY

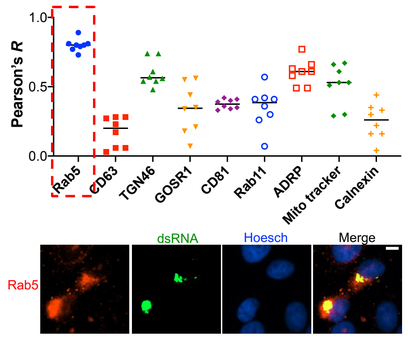
ABSTRACT

SARS-CoV-2 is the causative agent of the ongoing COVID-19 pandemic. While vaccines are readily available, the emergence of new SARS-CoV-2 variants along with the increasing incidence of individuals developing long COVID urge to develop antivirals specific to treat COVID-19. To reach this goal, a better understanding of the host-SARS-CoV-2 interactions is needed to identify targets for therapeutic intervention. With this in mind, we focused on studying the mechanisms by which SARS-CoV-2 generates replication organelles (ROs). All coronaviruses need to remodel cellular membranes to create these structures to allow the active replication and transcription of their genome. Due to their relevance for virus replication, disabling RO formation represents a promising strategy to fight SARS-CoV-2. However, the biogenesis mechanism, the origin, and type of these replication organelles are still a major focus of debate.

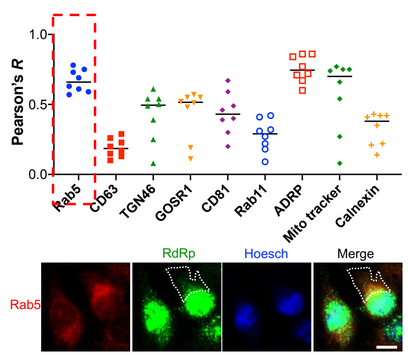
Here, we show that SARS-CoV-2 uses the viral protein NSP6 to remodel endosomal membranes and recruit them to perinuclear locations to generate replication organelles. Specifically, Clathrin, COPB1, and Rab5 are required for efficient SARS-CoV-2 RNA synthesis. This information opens new avenues to explore the endosomal pathway with the goal of developing strategies to interfere with this process and halt virus replication.

RESULTS

The SARS-CoV2 replication machinery co-localizes with endosomal membranes

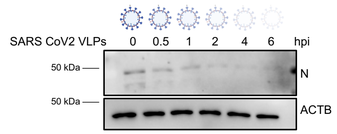


Fluorescence microscopy studies to locate the origins of SARS-CoV-2 ROs. Top panel: Pearson's correlation coefficient (*R*) value of intracellular markers (Rab5, CD63, Rab11, GOSR1, TGN46, CD81, Calnexin, ADRP, and mitochondria markers) and SARS-CoV-2 dsRNA. **Bottom panel:** representative pictures of the co-localization between the early endosomal marker Rab5 and SARS-CoV-2 dsRNA.

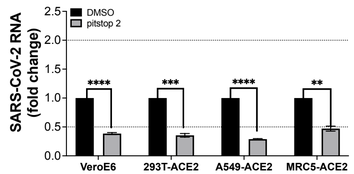


Fluorescence microscopy studies to locate the origins of SARS-CoV-2 ROs. Top panel: Pearson's correlation coefficient (*R*) value of intracellular markers (Rab5, CD63, Rab11, GOSR1, TGN46, CD81, Calnexin, ADRP, and mitochondria markers) and SARS-CoV-2 RdRp. **Bottom panel:** representative pictures of the co-localization between the early endosomal marker Rab5 and SARS-CoV-2 RdRp.

Clathrin is required for SARS-CoV-2 RNA synthesis

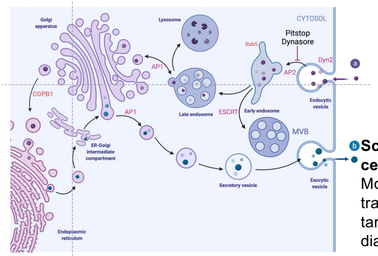


SARS-CoV-2 entry kinetics. Virus entry was examined to prevent a block in this first step of the virus replication cycle by endocytosis inhibitors. Entry was evaluated by synchronizing the infection at 4ºC and assessing the levels of the viral nucleocapsid (N) over time.

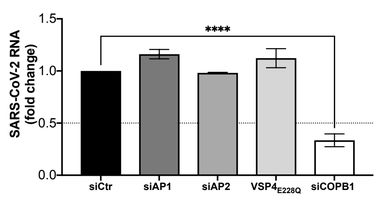


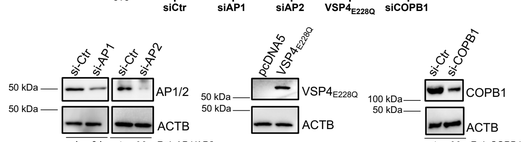
Blocking endocytosis causes a significant defect in viral RNA synthesis. The endocytosis inhibitor pitstop2 was added to infected cells 4.5 hpi. SARS-CoV-2 replication was measured by RT-qPCR.

COPB1 is required for SARS-CoV-2 RNA synthesis



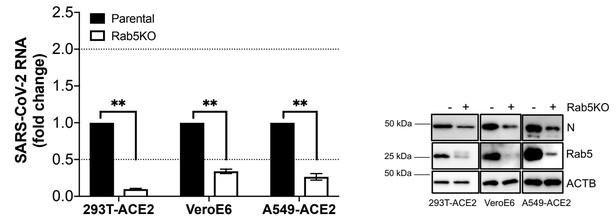
Schematic representation of cellular vesicular trafficking. Molecules involved in vesicular trafficking as well as drug targets are indicated in the diagram.





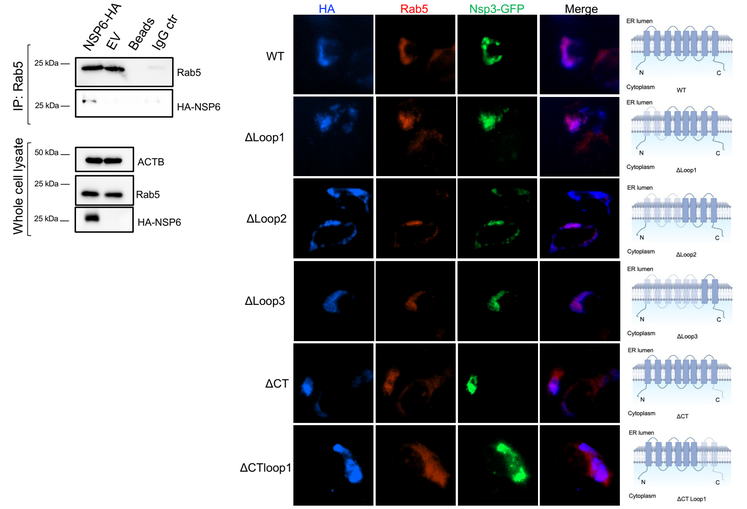
COPB1 depletion reduces significantly SARS-CoV-2 RNA synthesis. Top panel: Cells were treated with si RNAs for AP1, AP2, and COPB1 To block the ESCRT machinery, the VSP4 dominant-negative mutant $VSP4_{E228Q}$ was used. SARS-CoV-2 replication was measured by RT-qPCR. **Bottom panel:** siRNAs knock-down efficiency and $VSP4_{E228Q}$ expression were measured by western blot.

Rab5 is required for SARS-CoV-2 RNA synthesis



Implication of Rab5 in RO biogenesis. Left panel: The role of Rab5 in SARS-CoV-2 replication was measured by RT-qPCR in Rab5 KO (knock out) cell lines. **Right panel:** KO efficiency was assessed by western blot.

SARS-CoV-2 uses NSP6 to interact with Rab5 and remodel endosomal membranes



Role of NSP6 in RO formation and association with Rab5. Left panel: co-immunoprecipitation between NSP6 and Rab5 in 293T cells. **Right panel:** co-localization between Rab5 and NSP6 mutants.

CONCLUSIONS

- **SARS-CoV-2 remodels endosomal membranes to generate replication organelles.**
- **Clathrin, COPB1, and Rab5 are required for SARS-CoV-2 RNA synthesis.**
- **SARS-CoV-2 uses NSP6 to remodel endosomal membranes.**