Multiple pro-viral effects of the host protein G3BP in SARS-CoV-2 Infection.

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Introduction

Early in the COVID-19 pandemic, SARS-CoV-2 protein-protein interaction studies revealed an interaction between G3BP and the nucleocapsid (N) protein. The interaction is dependent on an ITFG-based motif at the N-IDR and G3BP NTF2L domain. The NTF2L features a long binding groove formed by two α-helices and two β-sheets, consisting of a 5.6 Å wide groove and a 3.5 Å narrow groove. The aromatic ring of N-F17 inserts into the aromatic cage at the center of NTF2L binding groove, stabilized by multiple π-stacking interactions. Meanwhile, the bulky hydrophobic side chain of N-I15 inserts into the small groove, coordinated by NTF2L residues L10, V11, and P6 (Fig. 1).

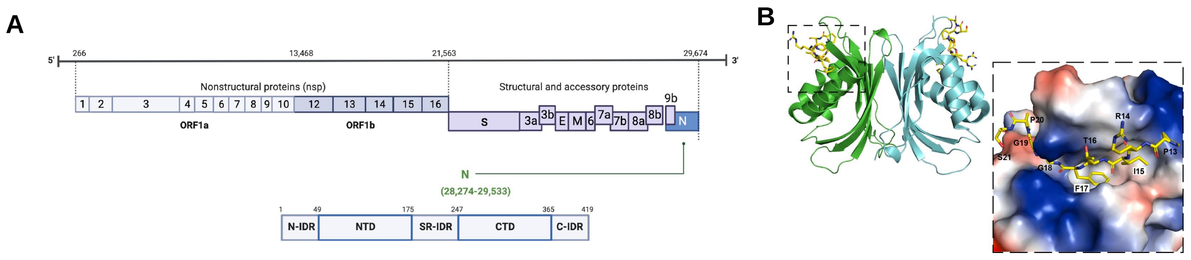

Figure 1. Overview of the interaction of SARS-CoV-2 N protein and G3BP. (A) SARS-CoV-2 genome and structural domain of N protein. SARS-CoV-2 gRNA is around 30 kb in size. ORF9 encodes N protein, which is composed of one NTD and CTD that are flanked by three IDRs, including a N-terminal IDR (aa 1–48), a central IDR (aa 175–246) that consists of a SR-rich region (aa 175–206) followed by C terminal IDR (aa 365–419). (B) Structure of G3BP1 NTF2 bound to N-WT (aa1-25) (PDB: 7SUO).

Results

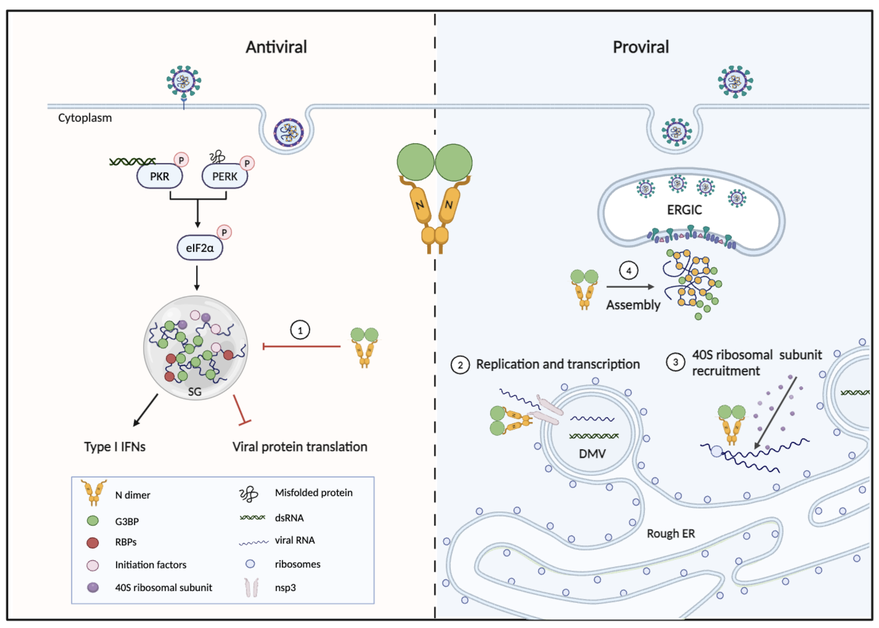

Figure 2. The antiviral and proviral roles of G3BP in SARS-CoV-2 infected cells. SARS-CoV-2 infection activated PKR/PERK-eIF2α serves as a protective mechanism in host cells, leading to the G3BP dependent SGs assembly. However, SARS-CoV-2 N protein hijacks G3BP, contributing to the enhancement of SARS-CoV-2 replication across multiple stages of the replication cycle: (1) G3BP-N interaction mediates the disassembly of SGs. (2) Early in infection, the N protein recruits G3BP to nsp3 at the RTC, potentially aiding in viral RNA synthesis and transcription; (3) The G3BP-N complex recruits 40S ribosomal subunits to viral factories for efficient viral protein translation; (4) G3BP promote the LLPS of N, facilitating SARS-CoV-2 virus assembly.

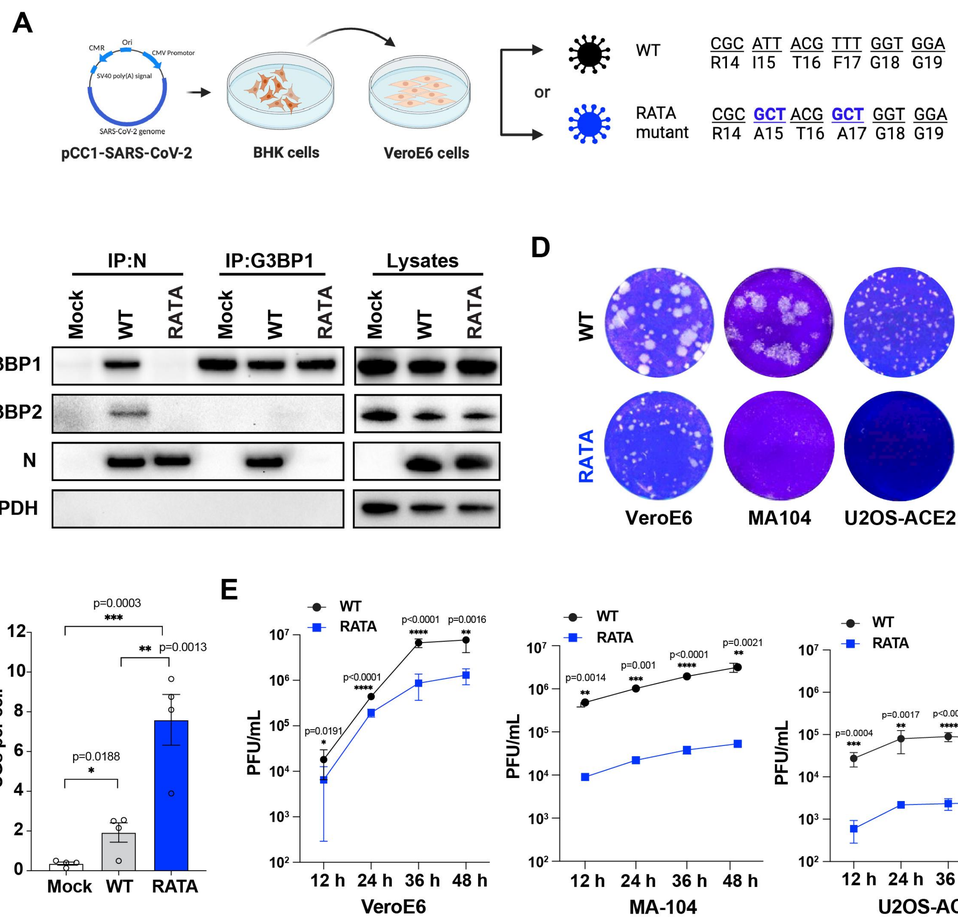

Figure 3. SARS-CoV-2 RATA is defective in SG inhibition and is attenuated in multiple cell lines. (A) Schematic of SARS-CoV-2 WT and RATA mutant. (B) VeroE6 cells were infected with WT virus or RATA mutant at 0.01 MOI for 24 h. Cells were lysed and immunoprecipitated with G3BP1 or N antibody for immunoblotting. (C) VeroE6 cells were infected with SARS-CoV-2 WT or RATA at 0.5 MOI for 6h. Quantification of SG foci was performed using CellProfiler. (D) Indicated cells were infected with SARS-CoV-2 WT or RATA until plaques were visible. Representative images show relative plaque sizes in indicated cell lines. (E) Viral titre from indicated cell lines infected with SARS-CoV-2 WT or SARS-CoV-2 RATA at 0.05 MOI.

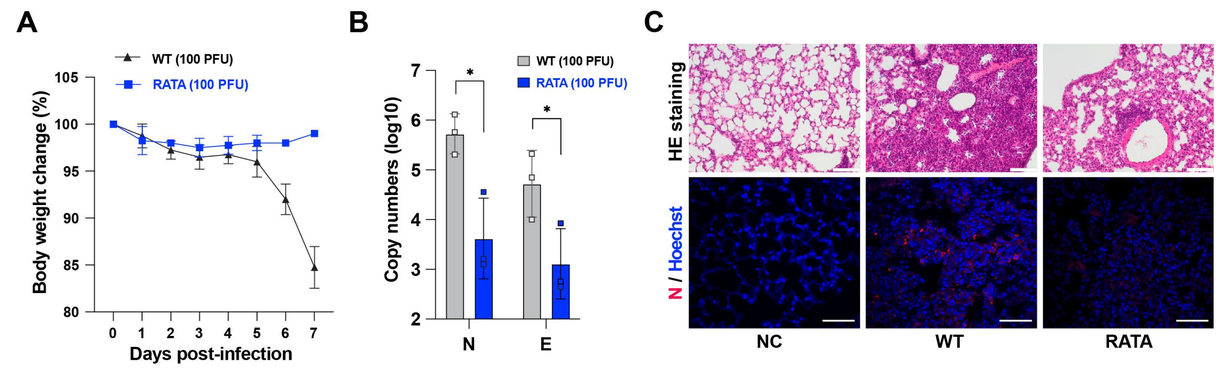

Figure 4. Infection of K18-hACE2 transgenic mice with SARS-CoV-2 WT or RATA mutant. (A) mice were inoculated with 100 PFU of SARS-CoV-2 WT or RATA and evaluated for weight loss (n=4 in each group). (B) RT-qPCR of N and E protein expression in mice lungs. (C) Lung histopathology and N protein immunohistochemistry staining in the lungs at 7 dpi from mock, SARS-CoV-2 WT and RATA infected mice. Scale bar = 50 μm.

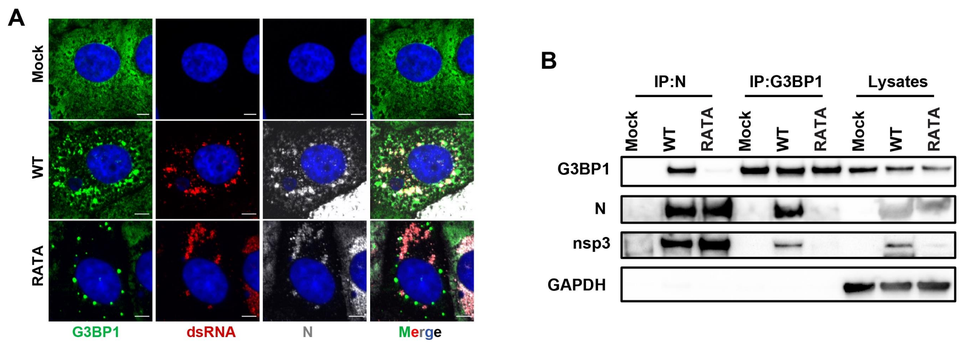

Figure 5. N recruits G3BP1 to RTC early in infection via interaction with pore protein nsp3. (A) VeroE6 cells were infected with WT SARS-CoV-2 at 0.5 MOI. Cells were fixed at 6 h and stained for G3BP1 (green), dsRNA (red) and N (grey), Hoechst (blue). Scale bar= 5 μm (B) U2OS-ACE2 cells were infected with WT virus or RATA mutant at 0.01 MOI for 24 h. Cells were lysed and immunoprecipitated with G3BP1 or N antibody for immunoblotting as indicated.

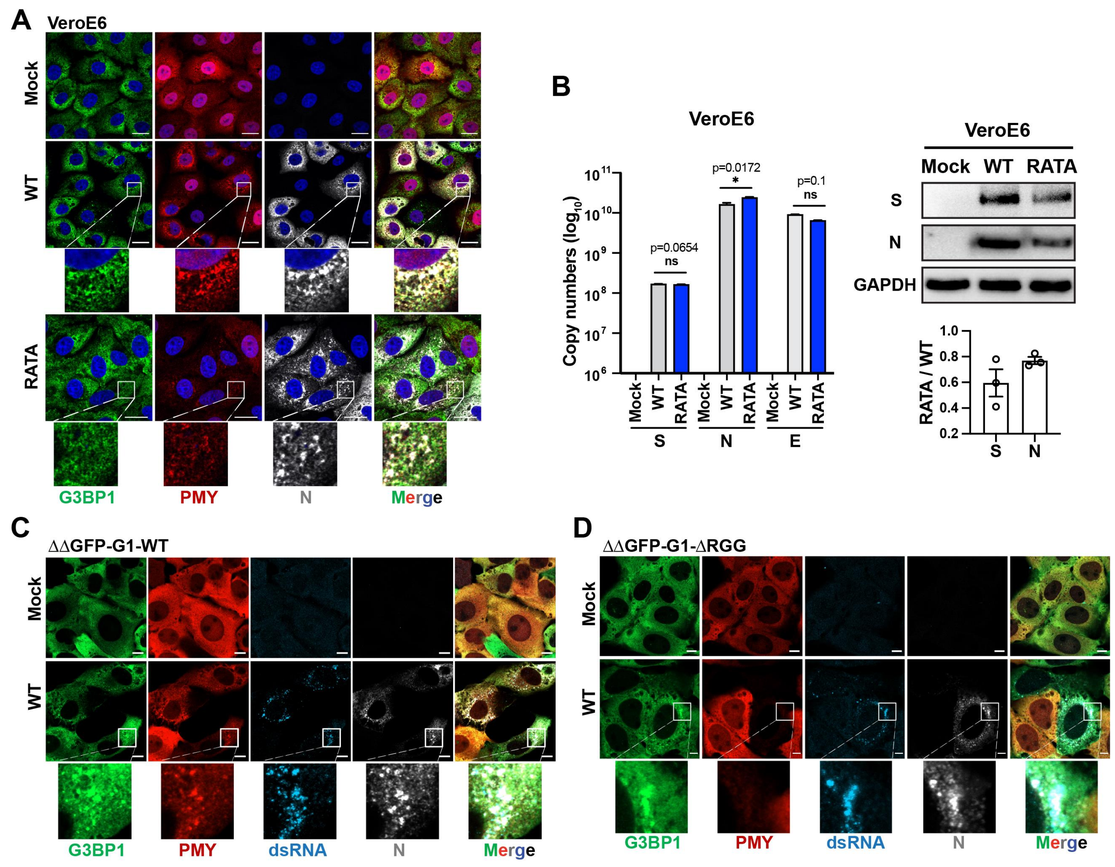

Figure 6. G3BP1 recruits 40S ribosomal subunit to viral factories. (A) VeroE6 cells were infected with WT or RATA virus at 0.5 MOI for 6 h. Cells were incubated with PMY (20 μg/mL) for 2 min before fixation and stained for G3BP1 (green), PMY (red), N (grey), Hoechst (blue). Scale bar = 20 μm. (B) VeroE6 cells were infected with SARS-CoV-2 WT or RATA mutant at 0.05 MOI for 6h, cells were collected for RT-qPCR to quantify viral RNA expression and for immunoblotting with indicated antibodies. (C-D) Indicated cell lines were infected with SARS-CoV-2 WT at 0.5 MOI for 6 h. Cells were incubated with PMY for 2 min before fixation and stained for PMY (red), N (grey), dsRNA (blue).

Conclusion

G3BP are multifunctional RNA-binding proteins, pivotal in the initiation of stress granules (SGs). SARS-CoV-2 N protein exhibits strong binding affinity for G3BP and inhibition of SG formation. However, pro-viral role(s) of the G3BP-N interaction have remained unclear. Here, we have comprehensively examined the importance of G3BP for SARS-CoV-2 infection both in vitro and in vivo. Using reverse genetics, we constructed a viral mutant, SARS-CoV-2 RATA, which exhibits stronger and more persistent SG response in infected cells. We also show that in SARS-CoV-2 infected cells, G3BP-N complexes are targeted to the pore complex of double membrane vesicles (DMV) from which nascent viral RNA emerges. Furthermore, through interaction with 40S ribosomal subunits, G3BP-N complexes promote highly localized translation of viral mRNAs at the viral factories and thus facilitate viral gene expression and replication. This work provides a mechanistic understanding of the roles of G3BP in SARS-CoV-2 infection.

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