SARS-CoV-2 Replicons Reveal Spike-Independent Attenuation of the Omicron Variant

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Introduction

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the coronavirus disease 2019 (COVID19) pandemic.
  - As of February 2023, more than 750 million people have been infected with and more than 6.8 million have died of COVID19.[1]
- Vaccination remains the primary strategy to curb the pandemic and has been shown to limit severe disease.[2] The emergence of variants represents a major threat to this strategy.
- The Omicron BA.1 variant was especially concerning due to large number of mutations across the entire genome.[3]
  - Associated with fewer symptoms and lower hospitalizations rates.[4]
  - Attenuated in cell culture and animal models of infection.[5]
- This apparent tradeoff between viral spread and infection severity in the context of an increasingly immunized population is poorly understood.
- **Hypothesis**: Mutations involving multiple viral processes contribute to the attenuated replicative phenotype of the SARS-CoV-2 Omicron BA.1 variant.

Golden Gate assembly enables rapid cloning of SARS-CoV-2 genomes

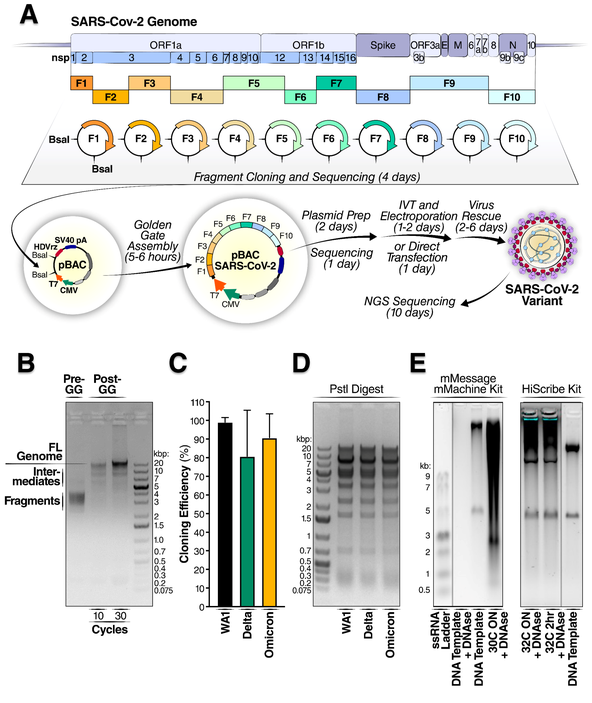


Figure 1. Rapid generation of SARS-CoV-2 variants. (A) Schematic of cloning methodology and generation of infectious clones. The estimated time required for each step is indicated in parentheses. (B) Agarose gel electrophoresis of Golden Gate (GG) assembly of the 10 fragments. (C) Cloning efficiency of SARS-CoV-2 variant infectious clones. Correct colonies are defined as those with perfectly correct sequence across the entire genome. 20–40 colonies were analyzed for each variant. (D) Agarose gel electrophoresis of PstI digest of 0.5 µg of SARS-CoV-2 variant infectious clone plasmids, demonstrating high quantity and quality of plasmid preps. (E) *In vitro* transcription of the WA1 assembled plasmid to generate full-length RNA under different conditions.

SARS-CoV-2 infectious clones replicate similarly to patient isolates

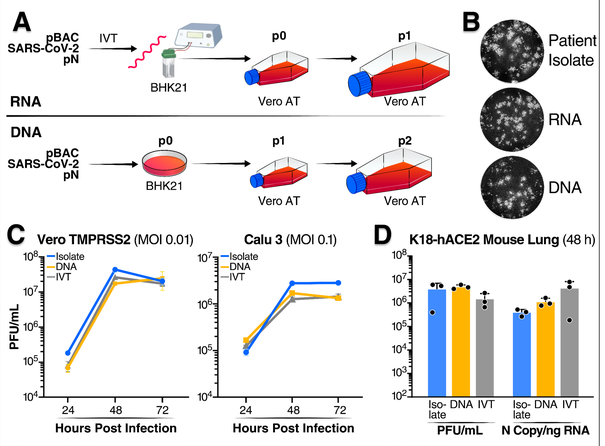


Figure 2. Comparison of RNA- and DNA-launched SARS-CoV-2 Delta variant to patient isolate. A) Scheme to produce RNA- and DNA-launched SARS-CoV-2. B) Plaque morphology of patient isolate, RNA- and DNA-launched Delta variant on Vero TMRPSS2 cells. C) Calu3 (MOI 0.1) or Vero TMPRSS2 (MOI 0.01) were infected with indicated viruses and virus release was measured by plaque assay (Vero TMPRSS2). D) K18-hACE2 mice were intranasally infected with 10^4 PFUs of indicated viruses and lung tissue was collected for plaque assay (Vero TMPRSS2) and RT-qPCR after 48 hours.

Omicron mutations in Spike and ORF1ab attenuate infectivity and replication

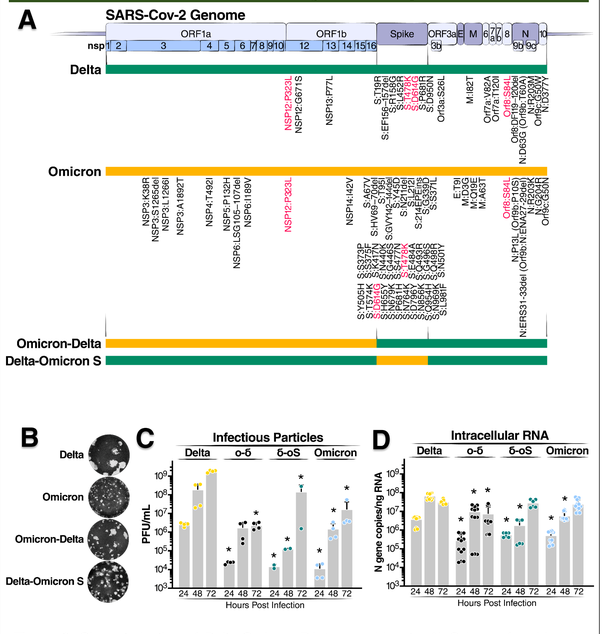


Figure 3. Characterization of Delta-Omicron BA.1 recombinants. A) Scheme for chimeric viruses construction. Mutations are representative of >90% sequences as of January 2022. Both recombinants have been observed in patients during the co-existence of both variants in late 2021. B) Plaque morphology of chimeric viruses in Vero ACE2 TMPRSS2 cells. Calu3 cells were infected with indicated viruses (MOI 0.1) and virus release was measured by plaque assay (Vero ACE2 TMPRSS2) (C) and intracellular RNA by RT-qPCR (D). *, p<0.01 by Student's T-test.

Replicon system to characterize Omicron RNA replication independent of Spike

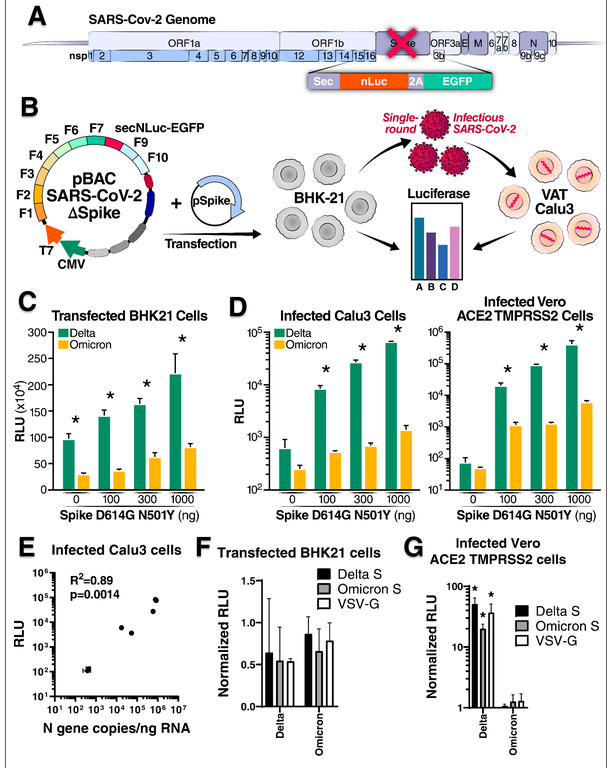


Figure 4. Assessment of Delta and Omicron variants' RNA replication levels. A) SARS-CoV-2 spike-independent replicon schematic. B) Workflow of a typical experiment utilizing the replicon system. BHK21 cells are transfected with the replicon plasmid and a spike expression construct. The supernatant is collected and filtered 72 hr post transfection and used to infect target cells, such as Calu3 and Vero ACE2 TMPRSS2 cells. Luciferase is measured in the supernatant of both transfected and infected cells 72 hr post transfection and infection, respectively, as a surrogate for viral RNA replication. C and D) Indicated replicon and spike expression constructs were analyzed as described in B. E) Calu3 cells were infected with serial dilutions of replicon supernatant and both luciferase activity in the supernatant and intracellular viral RNA were measured 72 hr post infection. F and G) Delta and Omicron replicons were transfected at 1:2 ratio with indicated envelope constructs and analyzed as described in B. *, p<0.01 by Student's T-test.

Omicron mutations in NSP6 lower viral RNA replication levels

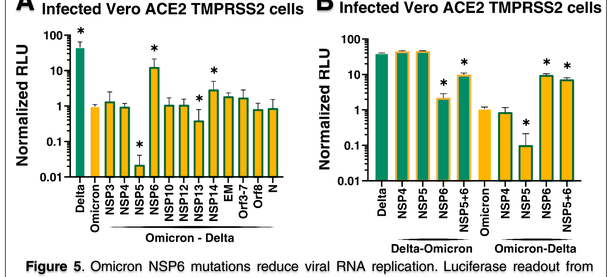


Figure 5. Omicron NSP6 mutations reduce viral RNA replication. Luciferase readout from infected Vero ACE2 TMPRSS2 cells with Delta, Omicron, and Omicron-Delta (A and B) or Delta-Omicron (B) recombinant replicons as indicated. *, p<0.01 by Student's T-test.

Omicron NSP6 has reduced LD consumption

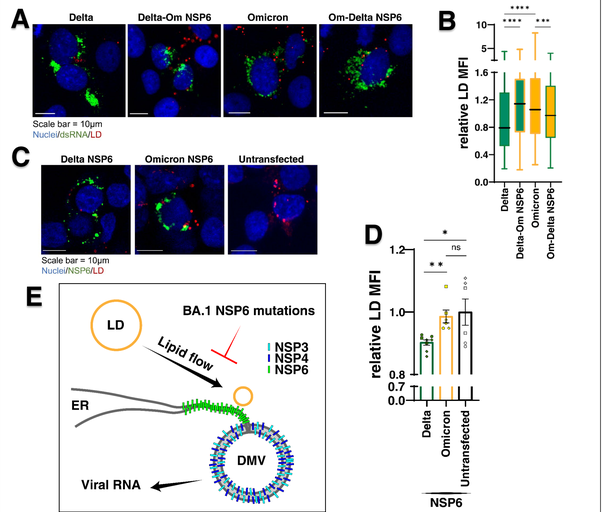
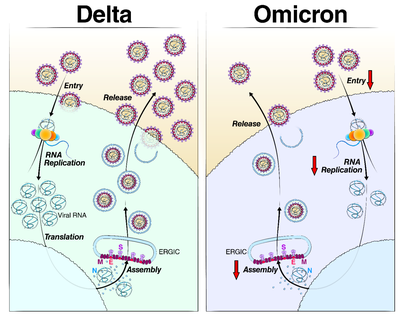


Figure 6. The Omicron NSP6 protein has reduced LD consumption. A) Delta, Omicron, and NSP6 recombinant replicons were used to infect Vero ACE2 TMPRSS2 cells and dsRNA and lipid droplets (LD) were measured by immunofluorescence and Lipidtox Red staining, respectively. B) Quantification of LD mean fluorescence intensity (MFI) in samples in A. C) Immunofluorescence analysis of NSP6 and LD in HEK293T cells transfected with indicated NSP6 expression vectors. D) Quantification of LD MFI in samples in C. E) Schematic of the role of NSP6 in channeling LD-derived lipids to double-membrane vesicles (DMVs) containing replication complexes. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001 by Student's T-test.

Conclusions and future directions



- Future directions include: 1) dissection of the mechanism by which Omicron NSP6 reduces viral RNA replication and 2) understanding the role of viral RNA replication as an evolutionary driver for Omicron subvariants.

References and notes

1. WHO; https://covid19.who.int/
2. Nat Rev Immunol. 2021 Oct;21(10):626-636.
3. GISAID; https://epicov.org/
4. Wolter N et al. Lancet. 399:437–446.
5. Suryawanshi RK et al. Nature. 2022 Jul;607(7918):351-355.

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- This work can be accessed at doi.org/10.1101/2023.01.31.525914

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