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Evaluation of Singer et al.: Technical Points on Analyzing Viral Replication Kinetics in Single Cells

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Abstract

One snapshot of the peer review process for “Quantitative measurements of early alphaviral replication dynamics in single cells reveals the basis for superinfection exclusion” (Singer et al., 2021).

Editor’s Note:

This is a first-round review of “Quantitative measurements of early alphaviral replication dynamics in single cells reveals the basis for superinfection exclusion” by Zakary S. Singer, Pradeep M. Ambrose, Tal Danino and Charles M. Rice; it was written for Cell Systems as part of the peer review process. We chose to feature it because the reviewers raised fundamentally important technical concerns that took a creative eye to find, and they raised them in an exceptionally constructive manner. After the first round of review, Singer et al. (2021) was revised to take the reviewers’ comments into account. The paper was then re-submitted, re-reviewed, accepted for publication, and published in this issue of Cell Systems. Xinyue Chen, Michael Pablo and Leor Weinberger blinded their identities during the peer review process but have chosen to reveal them here. Singer et al. support the publication of this Peer Review; their permission to use it was obtained after their paper was officially accepted. This Peer Review was not itself peer reviewed. It has been lightly edited for stylistic polish and clarity. Figure callouts refer to the figures in the original submission. No scientific content has been substantively altered.

This is a nice paper on alphavirus replication from an established lab with a long record of important contributions. Overall, the study provides well-presented data on the kinetics of early-strand synthesis in alphavirus using single-molecule and live-cell imaging, and offers a competition model for superinfection exclusion. We have a few technical concerns that should be addressed and specific writing that we hope will be helpful.

Technical Comments:

1. The fluorescent reporters tagged to nsP3 in Figure 4 are known to be efficient FRET pairs. How do the authors know that the anticorrelation observed is not due to FRET (mTurquoise2 serving as FRET donor for the mCitrine FRET acceptor)? FRET could generate the reduction in mTurquoise2 intensity and the correlation in panel G. nsP3 is proposed to multimerize in high-order structures (Schulte et al., 2016) and given the nsP3 punctal staining patterns in Fig. 3B at 5hpi, the authors should run a control to rule out FRET accounting for the data in Fig. 4G. Given the technical difficulties inherent to FRET detection, it might

be dangerous in this case to use absence of data (i.e., inability to detect FRET), as data for absence. However, one straightforward control is to simply repeat the experiment switching the order of the viruses in Fig. 4 with mCitrine virus first followed by mTurquoise2 virus as the superinfecting virus (i.e., putative FRET acceptor first followed by donor). If similar results are seen, then FRET could be ruled out. Given the centrality of this finding, this control seems important.

2. dsRNA and plus-strand RNA were quantified to indicate equal amounts of plus- and minus-strand synthesis during early infection (Fig. 2). This is a nice contribution but direct demonstration of minus-strand would be helpful. Could equal synthesis of both strands be directly shown if a similar time-course experiment was done in NaOH-treated cells (to break the duplex as in Fig. 2G)?

Specific clarification comments:

1. Fig. 1 would be of greater utility to the reader by providing a schematic of the competing models/hypotheses regarding strand synthesis and perhaps the expected outcomes of the analyses.

2. The authors mention single-cell approaches in virology. Some more discussion of the previous findings and specific contributions of single-cell analysis would be scholarly. Discussion of superinfection of bacteriophage and host-vs-virus programs in HIV seems relevant.

3. It would be helpful to show the representative traces of mTurquoise2 and mCitrine reporter viruses in the same cell analogous to Fig. 4E-F (i.e. add a new panel).

4. To explain the stereotyped logistic growth kinetics and distinguish an intrinsic virus-mediated program versus host shut-off program, the authors introduced point mutations in nsP2 and nsP3 and showed that the mutations didn't change the stereotyped kinetics (Fig. S2D). The mutant data is sufficient to show that the logistic kinetics is not dominated by host shut-off, but cannot distinguish intrinsic virus program versus host capacities for the viruses. This limitation should be discussed. It may help to contrast to bacteriophage and HIV in terms of host dominance (Razooky et al., 2015) (Zeng et al., 2010).

5. Please include the MOIs for Figures 2E, 2F, 2H, 3C-E.

6. There is a typo in the following sentence "tends" should be "tens": "Like a number of other RNA and DNA viruses across both prokaryotes and eukaryotes, Sindbis virus displays a superinfection exclusion phenomenon, wherein infection with one alphavirus can effectively block the replication of a subsequent alphavirus if introduced just tends of minutes later…."

7. In the sentence: "Based on the targeting of these probe sets to the sequence of the non-structural proteins, levels of subgenomic RNA will not contribute to their signal", please clarify the rationale. Indicate if non-structural proteins are generated exclusively from genomic rather than subgenomic RNA in this virus.

8. In the sentence: “This upper-bound in dsRNA abundance is likely a result of the accumulation of fully cleaved non-structural proteins within the replicase, which are unable to produce additional minus-strands”, clarify (provide citation to support) why proteins are expected to accumulate within the replicase.

9. The micrographs in all figures need scalebars.

10. Methods should state the type of regressions being conducted or the type of correlation coefficients being reported. Please clarify.

11. Figure 4D: What is “SE”? The panel title could perhaps simply be “superinfection exclusion quantification”, since all the work was done in BHKs.

12. The parameters for the Lotka-Volterra model are provided for MOI 5, 10, and “20” based on Fig. S3B. Perhaps the MOI “20” panel is a typo intended to be “30”, as the authors primarily focused on MOI 1/5/10/30. Additionally, the parameters are not shown for MOI 1 (and/or MOI 30). The model parameters should be listed or tabulated somewhere in the manuscript.

13. The figure call-outs to S3A in the model methods section should be corrected to S3B.

References

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