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Visualization of IAV Genomes at the Single-Cell Level

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Abstract

Different influenza A viruses (IAVs) infect the same cell in a host that can subsequently produce new virus through genome reassortment. By combining padlock probe RNA labeling with a single-cell analysis, a new approach effectively captures IAV genome trafficking and defines a time window for genome reassortment from same-cell co-infections.

Keywords

Visualization; Influenza A Virus Genomes; Single-Cell

Successful viral replication in a host depends largely on the susceptibility of host cells and the ability of the virus to replicate with them. Elucidating the cellular and molecular mechanisms of the viral infection process from entry and release will allow for the identification of novel targets that can be further explored toward development of new strategies for infection treatment and prevention. The infection process involves both virus and cell, and is very complex. Recently appreciated transcriptional and translational variability among different populations of cells or different subtypes even within the same populations [1, 2], collectively termed as cell-to-cell variability, further challenge experimental investigation toward fully elucidating the viral replication mechanism at the population level. Another challenge is the heterogeneous viral populations, especially among RNA viruses generated by error-prone replication. In the case of influenza A virus (IAV) infection, it becomes more complicated in cases where an infected cell harbors two different viruses, each consisting of eight gene segments with negative polarity. Segments between coinfecting viruses that encode the same set of proteins can undergo reassortments, thereby generating a new virus that can pose an immediate threat to humans. Therefore, gaining a full-spectrum knowledge of the viral infection landscape needs to take into account two variables: cell-to-cell variability as well as distinctive virus populations, which have driven the formation of a new trans-disciplinary approach, designated single-cell genomics for

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virology [3]. In recent years, new methods and technologies such as RNA sequencing (RNA-Seq) and single molecule-based fluorescence *in situ* hybridization (FISH) have been rapidly developed and employed to address how each individual cell responds to diverse populations of the virus [4–6]. The single cell-based measurement of viral infection may offer an advantage over cell population measurement especially in addressing the effect of cell-to-cell variability on viral infection.

Dou *et al.* recently developed a padlock probe (PLP)-based *in vivo* RNA labeling approach to study IAV genome replication and co-infection dynamics at the single-cell level [7]. The PLPs used in this study are linear oligonucleotides with IAV genome-specific recognition sequences distributed at their 5' and 3' ends. Upon hybridization to the IAV genome through PLPs' terminal sequences at the both ends that are designed to bind to two adjacent target sequences, PLPs become circularized by enzymatic ligation, which provides a template for synthesizing a rolling-circle product (RCP) [8, 9]. The ligation reaction is highly specific and will not occur if there is a single nucleotide mismatch between the target and the PLP at the ligation site. In that case, the mismatched PLP remains linear and its RCP will not be formed and amplified. Dou and colleagues improved this viral genome detection method by placing segment- and strain-specific barcode sequences between two terminal target recognition sequences in the PLPs. The addition of fluorescently labeled probes recognizing segment- and strain-specific barcode sequences into virus-infected cells can allow for the direct visualization of all eight IAV vRNA segments with the potential to identify the source of the virus. Following the validation of PLPs in interrogating IAV vRNA trafficking during entry and replication after genome release, the authors further advanced this tool with the labeling PLP as the readout to study IAV infection and genome replication in cells and lung tissue. Specifically, they conducted a spatiotemporal analysis of viral infections at both the single cell and the population levels by leveraging a wild-type (WT) IAV and its isogenic version containing a silent (synonymous) point mutation in each segment through PLP-labeling of viral segments individually and all together. In addition to monitoring the viral entry kinetics and detecting all eight segments semi-quantitatively, the authors found that PLPs could divide the early stage of virus lifecycle into three distinctive phases: cell entry, nuclear import, and genome replication. Remarkably, this methodology precisely distinguished cells co-infected by WT and mutant IAVs that varied only by a single nucleotide per segment. Another significant aspect of this work was that it provided temporal evidence that cell co-infections by two different IAVs leading to a productive genome reassortment only occurred when two different IAVs entered the same cell within a two-hour window. In summary, the multifunctional PLP labeling approach described in this study can be used as a powerful tool to investigate IAV replication dynamics at the single-cell level as well as to facilitate study of how mutations drive or restrict IAV genome segment reassortment.

The above study nicely complements a previous work by Heldt *et al.* who combined single-cell experiments with mathematic modeling to study cell-to-cell variability in IAV infection [10]. In that study, Heldt *et al.* demonstrated that each infected cell with the same cell type gave rise to variable amounts of infectious virus particles or viral genomes. For example, the authors found that there was substantial variability in vRNA expression levels between infected cells. They also noticed that the levels of individual vRNA segment varied within

the same infected cell. This cell-to-cell heterogeneity's effect was ultimately reflected in infectious IAV particle production in that viral yields differed by three orders of magnitude ranging from 1 to 970 infectious particles per cell. Finally, the authors observed that a significant amount of infected cells failed to produce viral particles, probably due to some defects occurring either in virus-cell fusion or vRNA transportation. The presence of defective interfering genomes may account for the failure to release viral particles by these nonproductive cells. Combination of the PLP labeling as described by Dou *et al.* with a single cell analysis may help to elucidate the mechanism as to why the majority of IAV-infected cells produced low amounts of infectious particles while some infected cells are highly productive [7, 10].

Similar to other high-resolution analytical approaches at this developmental stage, PLP has several shortcomings that include relative low throughput, limited sensitivity, and variable segment labeling efficiency. In addition, the spatiotemporal resolution of tracked viral genomes by PLP remains to be improved and live cell-imaging needs to be incorporated into next generation PLP methodology. Nevertheless, PLP labeling is emerging as a powerful tool that enables a single-cell analysis of IAV replication and coinfection dynamics with single-nucleotide resolution. Due to its versatility and specificity, it stands to reason that the PLP approach will soon be harnessed to study other RNA virus infections *in vitro* and *in vivo*.

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References

1. Buettner F, et al. Computational analysis of cell-to-cell heterogeneity in single-cell RNA-sequencing data reveals hidden subpopulations of cells. *Nat Biotechnol.* 2015; 33(2):155–60. [PubMed: 25599176]
2. Mingueneau M, et al. The transcriptional landscape of alphabeta T cell differentiation. *Nat Immunol.* 2013; 14(6):619–32. [PubMed: 23644507]
3. Ciuffi A, et al. Single-Cell Genomics for Virology. *Viruses.* 2016; 8(5):123.
4. Allers E, et al. Single-cell and population level viral infection dynamics revealed by phageFISH, a method to visualize intracellular and free viruses. *Environ Microbiol.* 2013; 15(8):2306–18. [PubMed: 23489642]
5. Grau-Exposito J, et al. A Novel Single-Cell FISH-Flow Assay Identifies Effector Memory CD4+ T cells as a Major Niche for HIV-1 Transcription in HIV-Infected Patients. *MBio.* 2017; 8(4):e00876–17. [PubMed: 28698276]
6. Josefsson L, et al. Single cell analysis of lymph node tissue from HIV-1 infected patients reveals that the majority of CD4+ T-cells contain one HIV-1 DNA molecule. *PLoS Pathog.* 2013; 9(6):e1003432. [PubMed: 23818847]
7. Dou D, et al. Analysis of IAV Replication and Co-infection Dynamics by a Versatile RNA Viral Genome Labeling Method. *Cell Rep.* 2017; 20(1):251–263. [PubMed: 28683318]
8. Lizardi PM, et al. Mutation detection and single-molecule counting using isothermal rolling-circle amplification. *Nat Genet.* 1998; 19(3):225–32. [PubMed: 9662393]
9. Nilsson M, et al. Padlock probes: circularizing oligonucleotides for localized DNA detection. *Science.* 1994; 265(5181):2085–8. [PubMed: 7522346]

10. Heldt FS, et al. Single-cell analysis and stochastic modelling unveil large cell-to-cell variability in influenza A virus infection. *Nat Commun.* 2015; 6:8938. [PubMed: 26586423]