Transcriptional dynamics of influenza virus infection at the single-cell level

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Abstract Influenza virus infection induces large changes in cellular transcription. Previously this has mostly been looked at using bulk measurements Here we examine the process at the level of single cells. We find extremely wide variation in the extent of viral gene transcription across infected cells. IFN induction is very rare. Some cellular pathways may be consistently altered in cells with high burden of viral transcripts. Overall, highlights remarkable heterogeneity in the outcome of infection.

Introduction

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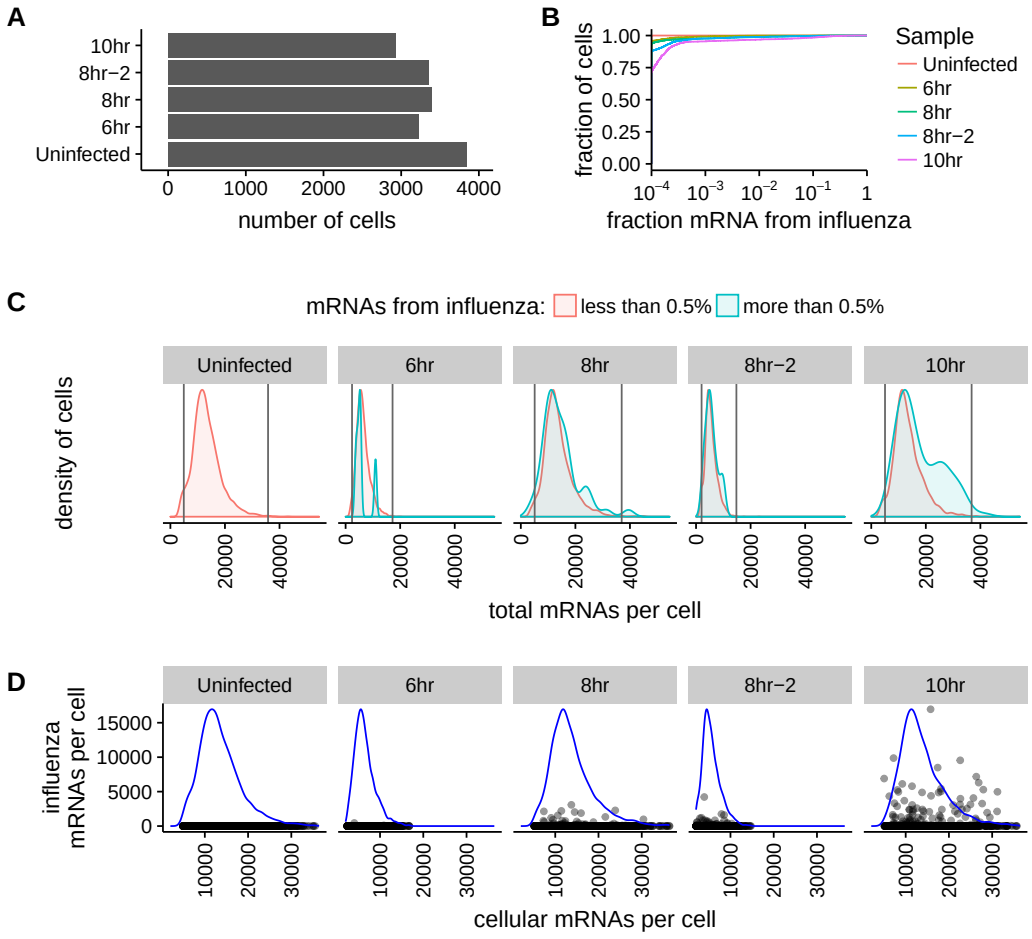

Figure 1. Overview of amounts of cellular and influenza virus mRNAs detected in each cell. **(A)** Number of cells captured for each sample. **(B)** Cumulative fraction plot showing the amount of mRNA derived from influenza for each sample. In all samples, most cells had little or no influenza mRNA. **(C)** Distribution of number of cellular and viral mRNAs per cell in each sample. Cells outside the vertical gray lines were considered outliers and excluded from subsequent analyses. **(D)** The number of cellular and viral mRNAs for each cell is plotted as a point. The blue lines show the overall distribution of the number of cellular mRNAs per sample. At later timepoints, a small number of cells had a very high number of viral mRNAs.

Figure 1–Figure supplement 1. Shorter caption for main text.

Figure 1–Figure supplement 2. This is another supplementary figure.

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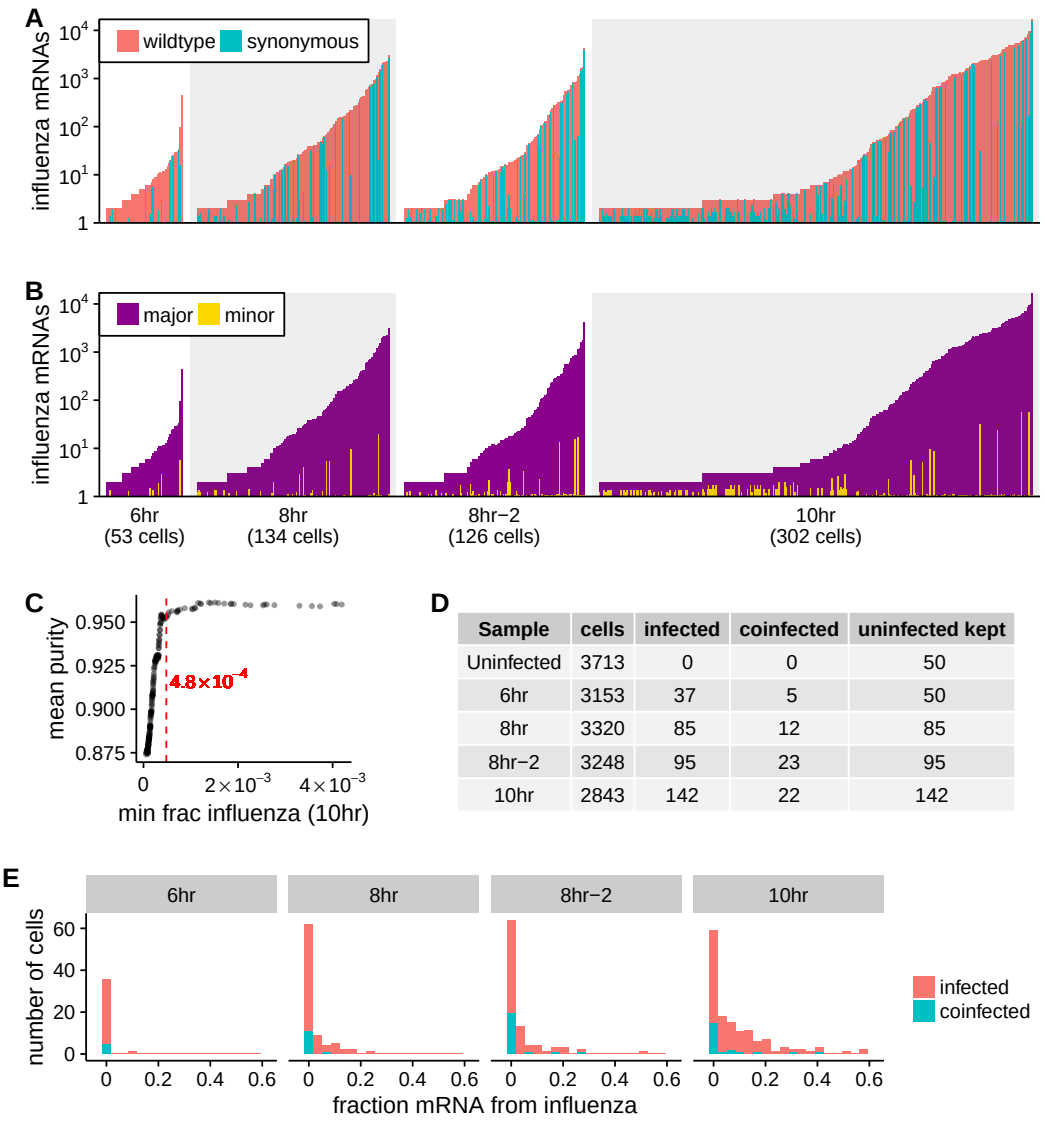

Sample	cells	infected	coinfected	uninfected kept
Uninfected	3713	0	0	50
6hr	3153	37	5	50
8hr	3320	85	12	85
8hr−2	3248	95	23	95
10hr	2843	142	22	142

Figure 2. Synonymous barcodes near the 3′ end of the influenza virus mRNAs were used to identify co-infection and distinguish true infections from cells that contained a few spurious viral reads. **(A)** For all cells with at least two viral mRNAs for which the synonymous barcode could be called, each line is proportional to the logarithm of the number of viral mRNAs in that cell. The bars are colored in linear proportion to the fraction of the viral mRNAs derived from either wildtype or synonymously barcoded virus. **(B)** Same as (A), but now each bar is colored according to the relative proportions of the more common (major) and less common (minor) barcoded virus variant. At low levels of viral mRNA there is often a roughly equal mix of barcodes, since many of these cells have simply picked up environment mRNA which is about equally likely to derive from either virus. But at higher levels of viral mRNA, truly infected cells are mostly one pure barcode except for a few cells that are truly co-infected. **(C)** We determined a cutoff for calling "true" infections by fitting a curve to the mean barcode purity of all cells with greater than a given fraction of their mRNA derived from virus. We called the cutoff at the point at which purity stops increasing with the fraction of viral mRNA. **(D)** The number of cells identified as infected and co-infected for each sample. For all samples, the vast majority of cells were not infected, so for subsequent analyses we subsampled to a number of uninfected cells that was the greater of 50 or the number of infected cells. **(E)** The distribution of the fraction of mRNA derived from virus for each sample for both infected and co-infected cells. For all samples, there is a very wide distribution of the amount of viral mRNA.

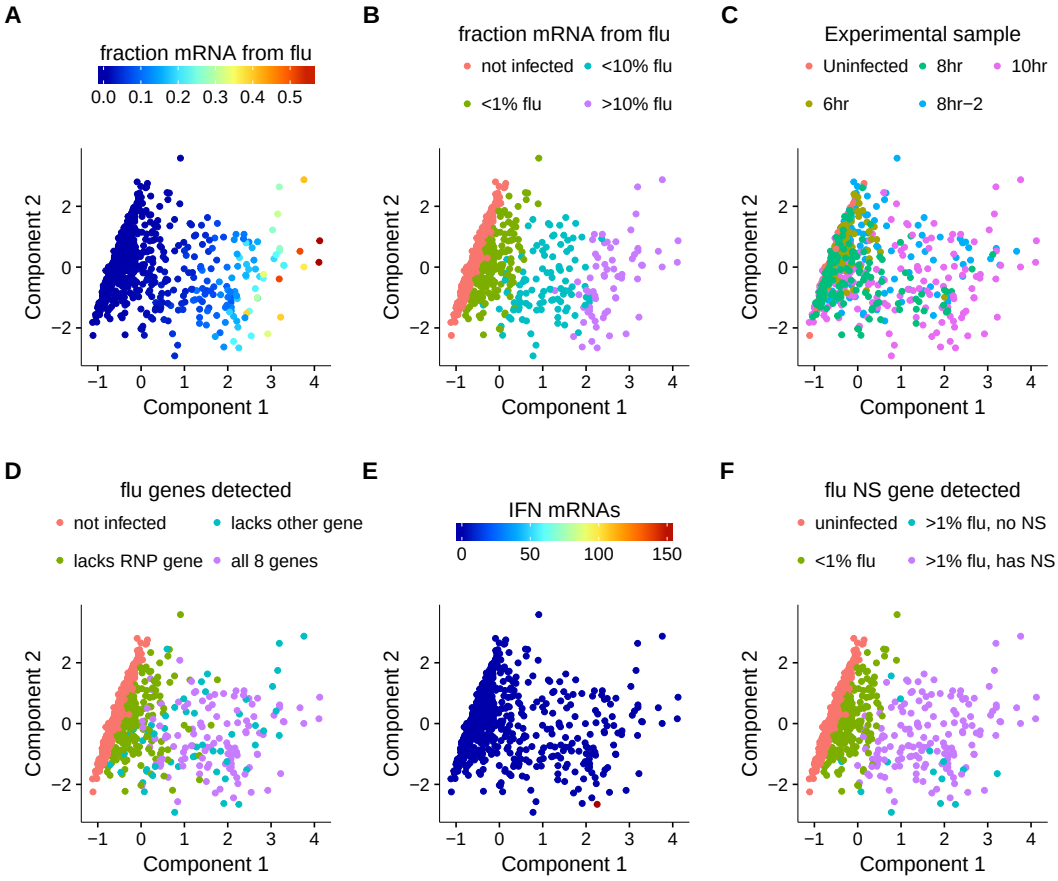

Figure 3. Visual layout of the cells according to "pseudotime". The layout is the same in all panels, but each panel colors the cells according to a different property. **(A), (B)** Cells colored by the fraction of their mRNA that is viral. **(C)** Cells colored by experimental sample. While it is clear that cells from later timepoints often have more viral RNA, there are cells from earlier timepoints with a high viral burden and cells from late timepoints with a low viral burden. **(D)** Cells colored by whether they lack at least one RNP gene, whether they have all RNP genes but lack another gene, or whether they have all 8 viral genes. **(E)** Cells colored by the number of type I and III interferon transcripts detected. Only one cell has high expression of these interferons. **(F)** For cells with at least 1% of their reads from influenza, are the cells expressing the viral NS protein? The one interferon-positive cell is lacking NS, but many other cells also lack NS but do not express interferon.

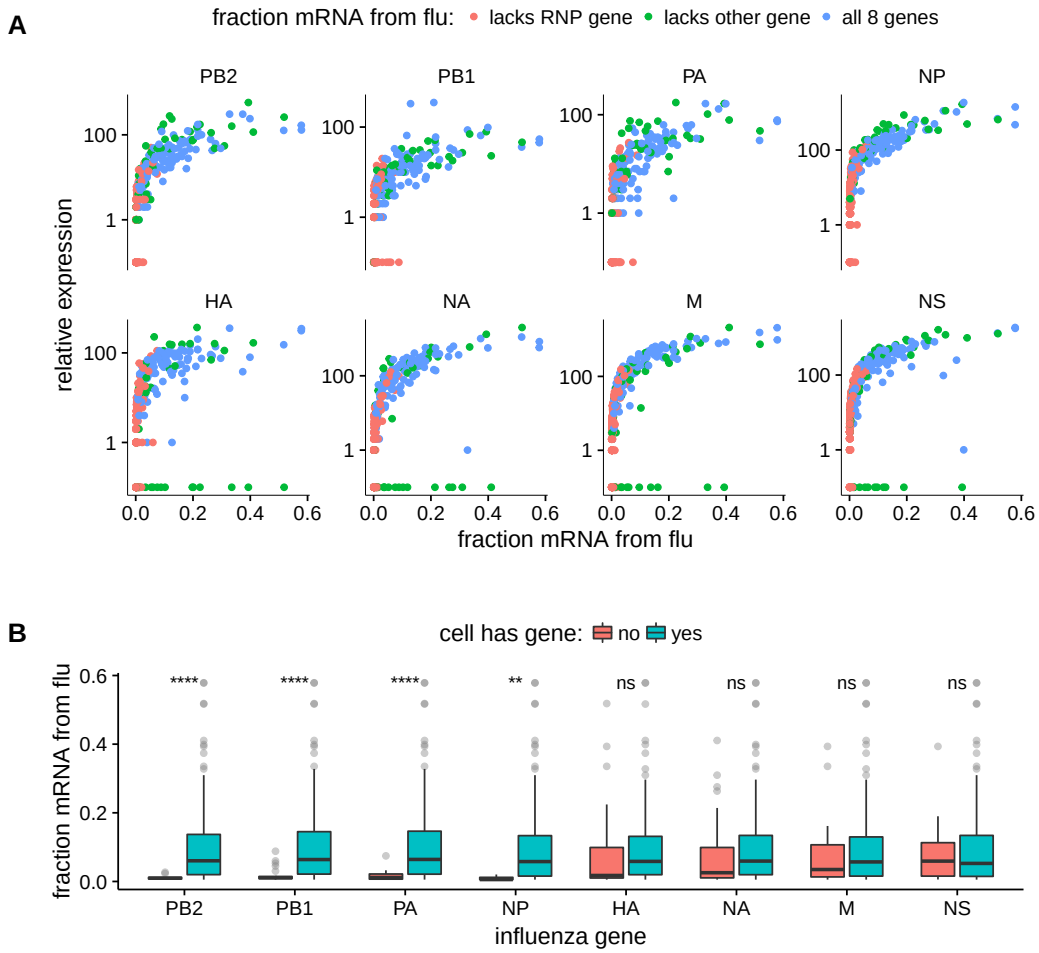

Figure 4. The viral infection burden in individual cells as a function of the amount of each viral gene detected. **(A)** Fraction of mRNAs in each cell derived from virus as a function of the *normalized* expression of each viral gene in that cell. This plot shows that all cells with very high viral burden express all of the RNP genes, but some cells with high viral burden lack each of the other four viral genes. **(B)** Statistical tests confirming that absence of viral RNP genes is significantly associated with reduced viral burden, but that the absence of the non-RNP genes does not lead to a clear decrease in viral burden.

Discussion

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Methods and Materials

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If you use the following prefixes for your `\label`:

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Tables `tab:`, e.g. `\label{tab:example}`
Equations `eq:`, e.g. `\label{eq:CLT}`
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you can then use the convenience commands as in `\FIG{cells}`, to generate cross-reference **??**.

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Acknowledgments

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References

Trapnell C, Cacchiarelli D, Grimsby J, Pokharel P, Li S, Morse M, Lennon NJ, Livak KJ, Mikkelsen TS, Rinn JL. Pseudo-temporal ordering of individual cells reveals dynamics and regulators of cell fate decisions. Nature Biotechnology. 2014; 32(4):381.

Figure 1–Figure supplement 1. This is a supplementary figure's full caption, which will be used at the end of the manuscript.

Figure 1–Figure supplement 2. This is another supplementary figure.