Key background references

Here we list some key background references on some relevant points.

Stochasticity during viral infection

- ***Delbrück*** (***1945***) showed that the amount of progeny virions released from bacteria infected with bacteriophage differed by over 2 orders of magnitude.
- ***Zhu et al.*** (***2009***) showed that the number of progeny virions produced by mammalian cells infected with VSV differ by over 2 orders of magnitude. They further suggest that the differences are mostly due to cellular factors, since they are not heritable.
- ***Schulte and Andino*** (***2014***) examine single cells infected with poliovirus. They found that the amount of both positive-sense and negative-sense genome and progeny virus differed by over an order of magnitude across cells. Higher MOI was associated with more positive-sense genome but *not* higher yields of progeny virus.
- ***Akpinar et al.*** (***2016***) examine VSV infection of single cells both in the presence and absence of defective interfering particles (DIPs). They track both the amount of produced viral progeny and fluorescence of a reporter. One interesting finding is that the viral yield from individual isolated cells is consistently lower than that from populations of cells, suggesting that presence of some cell-cell signaling or secreted factor might help viral production. They find that adding more DIPs decreases both viral production and the expression of the reporter gene, although the former is decreased more.

Influenza co-infection and defective particles

- ***Saira et al.*** (***2013***) shows that defective influenza particles are present in infected humans.

Interferon induction

- ***Shalek et al.*** (***2013***) and ***Shalek et al.*** (***2014***) using single-cell RNA-seq to show that interferon induction is highly variable across individual cells in bone-marrow derived dendritic cells. Some cells express lots of IFN related genes, others express almost none. They suggest that some "precocious" cells are responsible for much of the IFN signaling.
- ***Bhushal et al.*** (***2017***) look at expression of IFN in single cell using an RFP reporter. In epithelial cells, type I IFN induction is bimodal, with both the fraction of cells responding and the magnitude of response increasing with more IFNbeta, and saturating at near 100% response. In contrast, for type III IFN, although the response is also bimodal, it saturates at less than 100%, and there is less dose-response in the magnitude. They also did interesting experiments looking at "memory" in the response by separating responders and non-responders and then re-stimulating. Cells that did not express IFN first time still had a fraction the responded the second time, whereas responders the first time usually although not always responded the second time. So there is some but incomplete memory among responders. They show that at least for type III IFN, the difference between responders and non-responders is not at the level of STAT1 activation, but is rather downstream and may involve histone acetylation as it is affected by HDAC inhibitors. They also look in organoids and polarized cells, and find that in this more physiological setting the cells are more type III IFN responsive.
- ***López*** (***2014***) reviews the idea that defective genomes are important for IFN induction by many viruses including influenza.
- ***Baum et al.*** (***2010***) argues that RIG-I preferentially binds to short (defective) influenza segments.
- ***Tapia et al.*** (***2013***) shows that mice infected with influenza with more defective particles have more IFN induction.

Transcriptional dynamics

- ***Hatada et al.*** (***1989***) examine the accumulation of each influenza mRNA by Northern blot.

They find the same general hierarchy of expression as us: M and NS are the highest, then NP, then NA, then HA, then the three polymerases. Specifically estimates that the polymerase proteins reach about 10^3 copies / cell, HA gets to about 7×10^3 per cell, NA to about 10^4 per cell, NP to about 1.5×10^4 per cell, NS to almost 2×10^4 / cell, and M to over 2×10^4 cell.

- ***Kawakami et al.*** (***2011***) use qPCR to look at the accumulation of NP and NA in infected cells. They suggest that NP mRNA reaches a peak around 6 hours of 5×10^4 / cell, whereas NA reaches a much lower peak and after more time.
- ***Shapiro et al.*** (***1987***) examines gene expression using Northern blots. They find that the *rate* of mRNA synthesis (at least for NP, M1, and NS1) generally peaks around 2.5 hours, with protein levels appearing to be near maximal by about 5.5 hours. The rate of mRNA synthesis had fallen to 5% of the maximal rate by 4.5 hours. Note that these times are for infection intiated *after* absorption has been allowed to occur.

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