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.
- *Combe et al.* (*2015*) also look at the production of VSV from single infected cells, although immediately some of these cells are likely multiply infected. They find that the yield of viral progeny differs by about two orders of magnitude across cells.
- *Heldt et al.* (*2015*) examine stochasticity during influenza virus infection. They show that the amount of infectious progeny virions produced per infection (at high MOI) ranged from one to almost 1000. They also show that the amount of vRNA per cell (again at high MOI infection) ranges by almost 3 orders of magnitude. The amount of vRNA and progeny virions is strongly correlated. For most segments, the amounts of different mRNAs is correlated. They also have a bunch of simulations that I looked at less closely.
- *Dou et al.* (*2017*) use an RNA-labeling method to look at the presence of vRNAs in single infected cells. By their method, they find that only about 20% of cells contain all 8 vRNPs. They also show that there is a very limited window for co-infection.

Influenza co-infection and defective particles

- *Davis et al.* (*1980*) is an old paper that appears to be the original one showing that influenza defective RNAs have both termini and so represent some type of internal deletion.
- *Odagiri and Tashiro* (*1997*) look at a PA defective segment in the presence of an NS2 mutant. They show that the shorter defective PA segment is favored at both the levels of segment replication and genome packaging. I remain a bit unclear about how the NS2 mutant plays into all of this, other than that apparently this NS2 mutant generates lots of PA defectives...
- *Hutchinson et al.* (*2008*) look at how many infected cells lack expression of at least one of two segments tested. They find that for wildtype virus this is about 20%, but when there are synonymous mutations in the packaging signal of M, then it is about 50%.
- *Saira et al.* (*2013*) shows that defective influenza particles are present in infected humans.
- *Brooke et al.* (*2013*) show that over half of cells infected with PR8 fail to express one of four proteins tested. High MOI infection alleviates this incomplete expression, presumably via co-infection with viruses each of which fails to express different genes.
- *Brooke et al.* (*2014*) find a mutation in NP that specifically reduces the packaging of NA in a way that is advantageous in guinea pigs. They do various things showing that co-infection occurs *in vivo*, and also show that there is at least some amount of dose-response in terms of

49 gene expression as cells that get lots of no-NA virus during co-infection express relatively less
50 NA protein.

Interferon induction

52 • *Pérez-Cidoncha et al.* (*2014*) looks at type I IFN induction in A549 cells with a fluorescent
53   reporter. The wildtype virus induces IFN in a few percent of cells. A NS1-deletion virus
54   induces in 15-20% of cells. They passage in IFN-deficient cells to isolate various mutants that
55   induce more than wildtype but less than the delta-NS1 virus. Most of these mutations are in
56   proteins other than NS1, including some in M1 that were shown to induce IFN more effectively.
57   They also argue that some of the inducing mutations lead to more defective segments to
58   accumulate.
59 • *Killip et al.* (*2015*) reviews IFN induction by influenza, including the point that only a fraction
60   of cells induce IFN upon infection.
61 • *Killip et al.* (*2017*) look at IFN induction by wildtype and NS1-defective influenza virus. Although
62   some viruses (like Sendai) are able to induce IFN expression in almost all their cells, influenza
63   only induces IFN in less than one percent of cells. An NS1-defective virus induced IFN in
64   many more cells, but it was still only 5 to 20%. In neither case is there an obvious correlation
65   between NP protein production and IFN activation – IFN is both present and absent in cells
66   with little and lots of NP.
67 • *Shalek et al.* (*2013*) and *Shalek et al.* (*2014*) using single-cell RNA-seq to show that interferon
68   induction is highly variable across individual cells in bone-marrow derived dendritic cells.
69   Some cells express lots of IFN related genes, others express almost none. They suggest that
70   some "precocious" cells are responsible for much of the IFN signaling.
71 • *Bhushal et al.* (*2017*) look at expression of IFN in single cell using an RFP reporter. In epithelial
72   cells, type I IFN induction is bimodal, with both the fraction of cells responding and the
73   magnitude of response increasing with more IFNbeta, and saturating at near 100% response.
74   In contrast, for type III IFN, although the response is also bimodal, it saturates at less than
75   100%, and there is less dose-response in the magnitude. They also did interesting experiments
76   looking at "memory" in the response by separating responders and non-responders and then
77   re-stimulating. Cells that did not express IFN first time still had a fraction the responded the
78   second time, whereas responders the first time usually although not always responded the
79   second time. So there is some but incomplete memory among responders. They show that
80   at least for type III IFN, the difference between responders and non-responders is not at the
81   level of STAT1 activation, but is rather downstream and may involve histone acetylation as it is
82   affected by HDAC inhibitors. They also look in organoids and polarized cells, and find that in
83   this more physiological setting the cells are more type III IFN responsive.
84 • *López* (*2014*) reviews the idea that defective genomes are important for IFN induction by many
85   viruses including influenza.
86 • *Baum et al.* (*2010*) argues that RIG-I preferentially binds to short (defective) influenza seg-
87   ments.
88 • *Tapia et al.* (*2013*) shows that mice infected with influenza with more defective particles have
89   more IFN induction.

Transcriptional dynamics

91 • *Hatada et al.* (*1989*) examine the accumulation of each influenza mRNA by Northern blot.
92   They find the same general hierarchy of expression as us: M and NS are the highest, then NP,
93   then NA, then HA, then the three polymerases. Specifically estimates that the polymerase
94   proteins reach about 10^3 copies / cell, HA gets to about 7×10^3 per cell, NA to about 10^4 per
95   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.
96 *Vreede et al.* (*2004*) This paper examines production of mRNA and cRNA in the presence
97   of cycloheximide. They demonstrate the cRNA production requires the presence of the

polymerase proteins (PB2, PB1, PA, NP) in excess of those in the initial vRNAs, although the PB1 can be inactive.

- *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. The paper also shows that mRNA levels peak at 4-6 hours regardless of the presence or absence of cycloheximide, but the mRNA production is less in the presence of cycloheximide indicating secondary transcription.
- *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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