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Robust Kinetics of RNA Virus: Transcription Rates Are Set by Genome Levels

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Abstract

In order to persist in nature, RNA viruses have evolved strategies to grow in diverse host environments. To better understand how such strategies might work, we used qRT-PCR to measure viral RNA species during cellular infections by a model RNA virus, vesicular stomatitis virus (VSV). Absolute levels of the VSV major transcript and genome were measured for infections in BHK and PC3 cells, across different multiplicities of infection (MOI 1, 10, 100), in the absence or presence of protein synthesis, as well as in cells in an interferon-activated anti-viral state. While viral genome replication was delayed in more resistant host cells, kinetic modeling of these data revealed a simple linear relationship between the mRNA production rate and genome levels under all tested conditions. These results indicate that while viral transcription and genome replication both depend on the availability of the viral RNA-dependent RNA polymerase and host cellular resources, transcription proceeds without apparent limits on these resources.

Keywords

vesicular stomatitis virus; kinetics; transcription; replication; differential equations

Introduction

In order to be successful at infecting a wide range of cells types, virus growth must be robust to different host cell conditions. Variability in the host environment can have dramatic effects on the production of infectious virus particles (Timm and Yin, 2012; Zhu et al., 2009). Viruses may overcome such limitations by using robust strategies that are insensitive to host variability.

Vesicular stomatitis virus (VSV) has a broad cell tropism that makes it an attractive candidate as a vaccine vector, oncolytic agent, and gene delivery vehicle (Hastie et al., 2013; Lichty et al., 2004). For VSV, its surface glycoprotein (protein G) binds to the ubiquitous LDL receptor (Finkelshtein et al., 2013), enabling the virus to gain access to virtually any cell type. Viral entry/uncoating, gene expression, and assembly/release are further affected

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Supporting Information

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by a plethora of host cell factors (Panda et al., 2011). Moreover, virus growth is also sensitive to activation of host cellular anti-viral defenses (Carey et al., 2008; Stojdl et al., 2003) as well as the cell-cycle stage (Zhu et al., 2009). Despite this wealth of information on the multiple host factors that can impact virus growth, relatively little is known about how central processes of viral transcription, protein synthesis, and genome replication are coordinated or balanced in different host cell environments.

Non-segmented negative-sense (NNS) RNA viruses such as VSV have a unique transcriptional process, which is simplified in Figure 1. The viral particles contain the genome, polymerases, and necessary helper proteins to initiate primary transcription. Transcription initiates at the 3' transcriptional promoter so the gene most proximal to the 3' end is first transcribed (Rose, 1980). At each intergenic junction, the polymerase either initiates transcription of the next gene or does not initiate transcription, in which case the polymerase may dissociate from the template (Barr et al., 1997; Lyles and Rupprecht, 2007). By this way, a gradient of gene expression is produced depending on the position of the gene relative to the 3' end (Lyles and Rupprecht, 2007).

Primary transcripts are translated and the feedback of new viral proteins leads to genome replication (Davis and Wertz, 1982; Lyles and Rupprecht, 2007). Genome replication is performed using the same catalytic polymerase units (L and P proteins) as used for transcription. The presence of sufficient levels of viral N protein is required to initiate genome synthesis (Davis and Wertz, 1982). The N protein coats the nascent RNA produced by the polymerase such that full length RNA templates in the cell are all encapsidated by the N protein (Lyles and Rupprecht, 2007). Genome synthesis occurs through a full length positive-stranded template intermediate (Lyles and Rupprecht, 2007).

Previous studies have reported the transcriptional attenuation and polymerase elongation rates for VSV in vitro (Iverson and Rose, 1981). Computational models of VSV have used this and other transcriptional data to predict mRNA production rates as a part of a simulation of the larger viral infection (Hensel et al., 2009; Lim et al., 2006). The current model of NNS RNA virus transcription suggests that genomes should act independently during primary transcription, so the initial multiplicity of infection (MOI) should not affect the production rate of mRNA when appropriately scaled by genome numbers (Lyles and Rupprecht, 2007).

However, open questions on resource allocations remain. While we may be able to predict primary mRNA production, it is unknown if the feedback of new viral polymerases associated with secondary transcription will increase the mRNA production rate. It is also possible that the switch of polymerase activity from transcription to replication could bring about an observable decrease in mRNA production. Further, some transcriptional studies were performed in vitro (Iverson and Rose, 1981); due to the interactions among resources required for transcription, such as availability of templates, viral RNA-dependent RNA polymerase (RdRp), and pools of nucleotide triphosphates (NTPs), mRNA production rates in vitro may be different from rates in vivo, in either permissive or resistant host cellular environments.

In this work, we initially examined how the VSV mRNA production rate changes in the presence or absence of translation. New polymerase molecules produced during translation have the potential to bring about different rates of primary and secondary mRNA production. Further, translation also leads to genome replication which potentially competes with the transcription process by using the same active unit for polymerization. We also investigate how the host environment affects transcription by measuring mRNA production rates on permissive baby hamster kidney (BHK) cells or resistant human prostate cancer (PC3) cells. VSV grows efficiently on BHK cells, but has been shown to be inhibited in infections of PC3 cells (Ahmed et al., 2004). PC3 resistance to VSV has been attributed to an active immune response (Carey et al., 2008), so we also investigate mRNA production and genome replication in unstimulated and interferon (IFN) stimulated PC3 cells. To determine how the mRNA production rate is related to genomes, we employed a qRT-PCR assay to quantitatively measure viral nucleocapsid (N) mRNA and genomes. To facilitate analysis, we further developed a simple three reaction model to describe genome replication and test the relationship between viral mRNA synthesis and genome levels.

Methods

Cell Culture

Baby hamster kidney (BHK-21) cells were maintained in 10% fetal bovine serum (FBS, Atlanta Biologicals, Norcross, GA), 1% Glutamax I (Gibco, Grand Island, NY) in Eagle's minimum essential medium (MEM; CellGro, Manassas, VA). Prostate cancer cells (PC3) were maintained in 10% FBS (Atlanta Biologicals) in RPMI 1640 medium (Gibco). All cells were passaged every 2 or 3 days when they reached confluency.

Virus Culture

Wild-type (N1) VSV (Wertz and Perepelitsa, 1998) was passaged on BHK cells at MOI=0.001 to prevent formation of defective interfering particles. Stocks were centrifuged and filtered to remove cell debris. Stock concentration was $\sim 10^9$ PFU/mL.

Virus Infections

Infections were performed in reverse order such that a single harvest of sample would yield the entire time course of the infection. For example, to sample an 8- and 12-h time point, the virus was added 12 h before harvest-time, then again 8 h before harvest-time, and then cells were harvested simultaneously.

Cells were grown in a 96 well plate overnight to prepare for infections. At each time point, the cell supernatant was removed and viral infections were performed by adding 20 μ L stock virus (see above) or 10-fold dilutions in culture media supplemented with 2% FBS (Atlanta Biologicals). Virus was adsorbed for 5 min at 37°C, then the cells were rinsed with $\sim 50\mu$ L 37°C DPBS. One hundred microliter of media was added, and the infection was allowed to proceed at 37°C. Cells were infected in duplicate wells. In all cases, multiple 96 well plates of cells were used to minimize the effect of removing the cells from the incubator. Cells plated in three parallel wells were used for counting to determine an average number of cells per well.

Cycloheximide Treatments

Where indicated, cells were treated with 50 μ g/mL cycloheximide in 2% media. Cycloheximide (Fisher, Waltham, MA) was originally dissolved in DMSO at a concentration of 100mg/mL and the solution was diluted appropriately into 2% cell media. Media containing cycloheximide (or vehicle only control) was added to cells 30 min prior to infection. Media was removed and virus was added as above (virus inoculum did not contain cycloheximide). After the PBS wash, described above fresh media containing cycloheximide or vehicle only was added to cells.

IFN Treatments

Recombinant human IFN- β (PBL Interferon Source, Piscataway, NJ) was added to cell culture media at 1,000 U/mL. One hour prior to infection, cells were overlaid with media containing IFN- β or control media. Media was removed and virus was added as above (virus inoculum did not contain IFN- β). After the PBS wash, described above fresh media containing 1,000 U/mL IFN- β or control media was added to cells.

RNA Extractions

Prior to RNA extraction, infections were halted by placing the culture plates on ice. RNA extractions were performed using the RNeasy 96 kit (QIAGEN, Valencia, CA) with the vacuum protocol according to manufacturer's instructions. The 96-well extraction format allows for rapid parallel processing of the samples. Output RNA concentrations ranged from 2 to 20 ng/ μ L.

Reverse Transcription

Reverse transcription was performed using the GoScript Reverse Transcriptase system (Promega, Madison, WI). Gene specific primers for either N mRNA (ttccggatttgaggccttgtaga) or the VSV genome (atcctgctcggcctgagatacaaa) were used in parallel reactions from RNA samples. 3.15 μ L of undiluted RNA (see above) was mixed with 2 μ L of 10 μ M gene specific primer and incubated at 70°C for 5 min, then immediately transferred to an EtOH/ice bath for 5 min. Then, 1.85 μ L of 25mM MgCl₂, 0.5 μ L nucleotides (10 μ M each), and 0.5 μ L GoScript Reverse Transcriptase was added to a final volume of 10 μ L. Reactions were incubated according to manufacturer's instructions.

qPCR

qPCR was performed using (ttccggatttgaggccttgtaga) and (atccagtgaataccggcgagatt) for N mRNA and (atcctgctcggcctgagatacaaa) and (gggtggtgcgatccctaatttctt) for genomic RNA using the SsoFast Supermix (BioRad, Hercules, CA) on a C1000 thermal cycler (BIORAD, Hercules, CA). Two microliter of 1:5 diluted cDNA or plasmid standard was added to an 8 μ L reaction mix containing 5 μ L SsoFast Supermix, 1.2 μ L of 5 μ M each primer mix, and 1.8 μ L RNase free water. qPCR reactions were performed in duplicate. The qPCR standard was a purified plasmid containing the VSV genome. Primers were optimized to detect viral RNA by dilutions of extracted viral genomes or infected cell RNA. Amplification protocol: (i) 95°C for 45 s, (ii) 95°C for 5 s, (iii) 62.5°C for 10 s+plate read, and (iv) Goto 2 45x. Melt

curve conditions were 65–95°C in 0.5°C increments with 5 s step times and a plate read at each step.

qRT-PCR Assay Optimization

Primers were initially tested for single product amplicons by running amplified reactions on an agarose gel. Primers were temperature and concentration optimized using a dilution of a plasmid with a full genome VSV insert as a PCR standard. After primer and amplification conditions were optimized, we tested the ability of gene specific primers to detect dilutions of viral RNA over a background of cellular RNA to determine limit of detection and linearity of the reverse transcription step.

Conversion to Number Per Cell

The starting quantity of the plasmid standard was determined by measured DNA concentration on the NanoDrop 200c (Thermo Scientific, Wilmington, DE) and dividing by the molecular weight of the plasmid to convert concentration to copy number. Infection samples were measured using the qRT-PCR assay described above. The starting quantity per well was converted to copy number per cell using the following equation:

$$\frac{\text{copies}}{\text{cell}} = \frac{\text{Starting Quantity}}{\text{qPCR well}} \cdot \frac{\text{qPCR well}}{\text{V uL cDNA}} \cdot \frac{\text{W uL cDNA}}{\text{X uL RNA}} \cdot \text{Y uL RNA} \cdot \frac{\text{mock RNA conc}}{\text{this RNA conc}} \cdot \frac{\text{well}}{\text{Z cells}}$$

Model Solution and Data Fitting

The following reactions and resulting differential equations were used as the model of VSV infection.

$$g^k m + g, k = \begin{cases} BHK & PC3 & condition \\ k_t & k_t & t < \tau \\ k_t & 0.06k_t & t \geq \tau \end{cases}$$

$$2g^{k_d} 0$$

$$g^{k_{rep}} 2g, k_{rep} = \begin{cases} 0 & t < \tau \\ k_r & t \geq \tau \end{cases}$$

The reactions are transformed into differential equations assuming mass-action kinetics, with discontinuous rate constants following the rules above.

$$\begin{aligned} \frac{dm}{dt} &= k_t g \\ \frac{dg}{dt} &= -2k_d g^2 + k_r g \\ g(0) &= g_0 \\ m(0) &= 0 \end{aligned}$$

The stated initial condition g_0 , transcription constant k_t , genome degradation constant k_d , genome replication constant k_r , and replication delay τ were fitted parameters.

The models used were implemented in MATLAB and solved using an open source software parest, [<http://jbrwww.che.wisc.edu/software/parest/>]. We used parest to minimize the likelihood function as defined by the difference between the log of the model solution and the log of the qRT-PCR measurement (in copies/cell), which is equivalent to maximizing the likelihood function. Parameter values which minimize the squared error objective function are reported as parameter estimates. Details of the procedure can be found in Rawlings and Ekerdt (Rawlings and Ekerdt, 2002).

Significance Testing for Model Parameters

In cases where statistical tests were performed on parameter values, we used a standard t -test treating parameter values as independent measurements.

Models were compared for significant improvement in sum-of-squared errors using the F -test (Wu and Hamada, 2009).

Results

Development of a qRT-PCR Assay to Measure the Kinetics of Viral N mRNA and Genomes During Infection

qRT-PCR primers were designed to target viral N mRNA as a proxy for all transcripts and genomic RNA as described in the Methods. We chose the N mRNA to act as an indicator of viral transcription, as it is the first transcribed gene and attenuation has been investigated elsewhere (Iverson and Rose, 1981). For each target, a single primer was used in the reverse transcription step: for N mRNA, the primer targeted the positive sense mRNA; for the genomic RNA, the primer targeted the negative strand. We took extra precaution to ensure that the primers could linearly detect viral mRNA and genomes in cellular samples. To do this, we diluted infected cell RNA (12 hpi, BHK cells) into mock-infected RNA to determine a lower limit of detection and establish that the primers detect RNA linearly over a biologically relevant range of conditions, as shown in Figure S1. These measurements represent averages from $\sim 10^5$ cells.

Measuring rates of mRNA production and replication require multiple time points with replicates over an appropriate time range for the experiment. Briefly, cells were infected at multiple times before harvest allowing a full 96 well plate to be collected and analyzed simultaneously. Using this technique, we were able to generate data sets with eight time points in duplicate for six conditions in a 96-well format.

Rate of mRNA Production Depends Linearly on Genome Levels in the Absence of Translation

Figure 1 outlines the processes of transcription and replication in VSV infections. As shown, the replication process depends on the synthesis of new viral N protein from primary transcripts. Viral mRNA and genomes during infection of cycloheximide-treated BHK cells at three initial MOI are shown in Figure 2. Viral genomes degraded with first-order kinetics

over the 6-h infection period. During this time, mRNA was produced linearly from genomes. Using a model based only on genome levels (and not protein levels), we are able to fit mRNA production data. Further, the rate constants between the three MOI conditions do not follow an observable trend and were of similar magnitude, suggesting that the primary mRNA production rate from genomes is independent of MOI.

Rate of mRNA Production Depends Linearly on Genome Levels in the Presence of Translation and Replication

BHK cells were infected using 10-fold dilutions of initial MOI. As shown in Figure 3, genome replication began after a delay that increases with decreasing MOI, and genome replication was essentially complete after 6 h of infection. mRNA production is observed immediately and continued throughout the 6-h infection. The mRNA levels over time were described using a model that is only based on genome levels, while the genome levels could be described empirically using an exponential replication reaction, a second order decay reaction, and a time delay. We chose this model structure as it yielded the minimum sum-of-squares error when compared to similar models, and the linear transcription from genomic RNA was consistent with the model fitting for cycloheximide treated cells.

Primary mRNA Production Rate is Slower Than Secondary mRNA Production Rate in PC3 Cells

We challenged the virus by infecting PC3 cells using 10-fold dilutions of MOI. PC3 cells have shown to be resistant to VSV in culture due to inhibition at multiple steps (Carey et al., 2008). As shown in Figure 4, we observed a long delay (2.3–3.6 h) before genome replication; the delay was longer at lower MOI. mRNA data during early infection for low MOI (Fig. 3C) was below the qRT-PCR detection limit and omitted from the plot and analysis. During the delay, mRNA production was slower than following the delay, behavior that was also observed in infections treated with cycloheximide (Figure S3). To model this behavior, we set the primary mRNA production rate constant to be a fraction (α) of the secondary rate constant as shown in the equations in Figure 4B. We estimated α to be 0.06, consistent with previous findings (Carey et al., 2008). In this context, α represents the fraction of genomes that are available for transcription while the balance of genomes remain in early endosomes in PC3 cells (Carey et al., 2008). The inclusion of this extra parameter was tested and found to significantly decrease the sum-of-squares error between model and data ($P < 0.001$, F -test).

Stimulation of the Innate Response by IFN Does Not Affect the Dependence of mRNA Production on Genome Levels

Mechanisms for PC3 resistance to VSV infection arise from a functional innate immune response (Ahmed et al., 2004; Carey et al., 2008) that is activated upon detection of viral intermediates through a variety of receptors. Activation of receptors stimulates innate response genes and the production of the anti-viral cytokines. The cytokines, which include type I interferons, act in positive feedback to produce a strong and fast innate response to viral infection (Faul et al., 2009). We chose to investigate how the innate response affects viral mRNA production and replication further by stimulating PC3 cells with 1,000 U/mL of interferon- β (IFN- β). IFN pretreatment has been shown to inhibit VSV at the level of entry

(Whitaker-Dowling et al., 1983) and primary transcription (Staeheli and Pavlovic, 1991). Figure 5 shows the data and model fits for mock-treated and IFN-treated cells. IFN stimulation did not affect genome delivery to cells as evidenced by similar fitted initial conditions. The mRNA production rate and genome replication rate were decreased by IFN treatment while the delay and degradation constants were increased by IFN treatment. Interestingly, the high MOI infection was better able to overcome the inhibitory effects of IFN as compared to medium or low MOI infections. The relative levels of mRNA and genomes decline more rapidly for medium and low MOI infections after 6 h whereas the relative levels for high MOI stay higher.

Discussion

VSV is known for its broad cell tropism (Lichty et al., 2004). In order to grow in multiple cell types under multiple conditions, we hypothesized that features of the VSV infection strategy would be relatively insensitive to host cellular environment. To test this hypothesis, we measured viral mRNA and genome kinetics under a variety of infection conditions. We used a lumped modeling approach to help analyze and draw conclusions from the detailed kinetics of the infection. Our model accounted for the production of genomes and mRNA as well as genome decay and delays in genome production. The ability of this simple model to fit experimental data shows a predictable relationship between genomes and mRNA and supports the hypothesis that mRNA is produced linearly from genomes early in infection. The results presented here suggest that the virus is operating in an environment with excess polymerase, as the polymerase levels do not need to be included in the equations to fit the mRNA production data.

We did not observe a decrease in the rate of mRNA production upon initiation of translation and genome replication. Because the same polymerase complexes are used to generate both mRNA and genomic templates, we might expect a shift to purely replication. The continued high mRNA production rate suggests that even in the presence of N protein levels sufficient to sustain replication, transcription is still the dominating role of the polymerases. This could be explained by a strong transcription promoter relative to replication or by a localization bias towards transcription due to formation of inclusion bodies (Heinrich et al., 2010).

Primary mRNA production occurred throughout the long cycloheximide treatment in both BHK and PC3 cells (Fig. 2A). Given a VSV processivity rate of 3.7 nt/s and a genome length of 11,161 nt, the residence time of a given polymerase on a genomic template is ~54 min. With a polymerase loading of 50 polymerases per genome (Thomas et al., 1985), we expect that the last polymerase would dissociate from the genome after 2 h into the infection. As shown in Figures 2A and S3, we see mRNA production that continues at a constant rate for up to 8 h in PC3 cells. Continued mRNA production suggests that polymerases are recycled after intergenic attenuation or reaching the end of the genome, as opposed to diffusing freely away from genomes. This hypothesis is supported by a loop-like structure observed in influenza RNA that would facilitate polymerase recycling (Arranz et al., 2012; Coloma et al., 2009).

Previous work has shown that polymerases can be inhibited or degraded by human MxA, known to be expressed in PC3 cells (Staeheli and Pavlovic, 1991). However, we do not see a continuing decrease in PC3 primary mRNA production which would be evidence of continued degradation of polymerases. It is possible that they are degrading but without a detectable large effect on the transcript production rate.

MOI does not appear to have a large effect on mRNA production rates as evidenced by the model fit to all MOI conditions. The lack of any trend in mRNA production rate constants with MOI suggests that genomes act independently during transcription, especially during primary mRNA production. However, we did observe some trends with MOI for other model parameters. In general, the delay before replication increased with a decrease in MOI, especially in PC3 cells. An explanation for shorter delay at higher MOI is that more genomes can make more mRNA and thereby establish a replication environment more quickly. We also observed a general decrease in the replication rate constant with decreasing MOI in PC3 cells. This may be explained by early innate response events which lead to a less-permissive replication environment. The IFN treatment helps support this by showing that the high MOI infections are able to overcome the innate stimulation better than the medium or low MOI infections. While the reactions used to model genome kinetics are not mechanistic, the rate constants do provide a summary of the data and allow us to measure a bulk rate of genome replication in the system.

Interestingly, the high MOI case was able to grow similarly in the IFN treated and untreated infections (Figs. 5 and S5). The high MOI rate constants for all reactions were similar in IFN treated and untreated infections, while in medium and low MOI, the degradation and replication constants were altered by the treatment. The results presented here show that it is feasible for the virus to overcome an innate response by initiating infections with large amounts of virus.

It is important to note that the model did not fully capture all trends in the data, most notably the saturation of the mRNA data after ~6 h in BHK infections and ~20 h in PC3 infections. Data after these times are lower than the model, highlighting a limitation of the model at later time in the infection. The saturation of mRNA data observed in longer infections is most likely explained by matrix protein inhibition of polymerase activity seen in other related viruses (De et al., 1982; Hankins et al., 1990; Suryanarayana et al., 1994). Other explanations include a decrease in available polymerases, nucleotides, or genomes available for transcription due to packaging steps. We chose not to include a mechanism for saturation of mRNA because most of the experimental data was before observable mRNA saturation. This means that the model presented here is more applicable for early infection kinetics and could be advanced by including more details of the later infection process. Future models coupled with quantitative protein kinetics will help extend predictions to all aspects of the virus infection.

We were able to capture the mRNA production and genome replication data using a relatively simple model of only three reactions. The model only uses genome levels and does not depend on protein concentration in the cells to fit mRNA production data. This suggests that the VSV replication strategy is constant even in the presence of an anti-viral response:

the virus establishes an environment of excess protein such that the mRNA production rate is constant from genomes. Being able to approximate the transcription process with this simple model does not suggest that the details of the transcription mechanism are not important. It does, however, provide a template for estimating the quantitative effects of mutations and drugs on the early infection process as evidenced by the IFN treatment experiment in Figure 5. Further experiments can be used to investigate other drug effects or the infection process in different cellular environments. Quantitative protein data and particle production data can also be incorporated to generate a full quantitative description of the viral infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Ahmed M, Cramer SD, Lyles DS. Sensitivity of prostate tumors to wild type and M protein mutant vesicular stomatitis viruses. *Virology*. 2004; 330:34–49. [PubMed: 15527832]
- Arranz R, Coloma R, Chichón FJ, Conesa JJ, Carrascosa JL, Valpuesta JM, Ortín J, Martín-Benito J. The structure of native influenza virion ribonucleoproteins. *Sci*. 2012; 338:1634–1637.
- Barr JN, Whelan SP, Wertz GW. Role of the intergenic dinucleotide in vesicular stomatitis virus RNA transcription. *J Virol*. 1997; 71:1794–1801. [PubMed: 9032308]
- Carey BL, Ahmed M, Puckett S, Lyles DS. Early steps of the virus replication cycle are inhibited in prostate cancer cells resistant to oncolytic vesicular stomatitis virus. *J Virol*. 2008; 82:12104–12115. [PubMed: 18829743]
- Coloma R, Valpuesta JM, Arranz R, Carrascosa JL, Ortín J, Martín-Benito J. The structure of a biologically active influenza virus ribonucleoprotein complex. *PLoS Pathog*. 2009; 5:e1000491. [PubMed: 19557158]
- Davis NL, Wertz GW. Synthesis of vesicular stomatitis virus negative-strand RNA in vitro: Dependence on viral protein synthesis. *J Virol*. 1982; 41:821–832. [PubMed: 6284973]
- De BP, Thornton GB, Luk D, Banerjee AK. Purified matrix protein of vesicular stomatitis virus blocks viral transcription in vitro. *Proc Natl Acad Sci U S A*. 1982; 79:7137–7141. [PubMed: 6296818]
- Faul EJ, Lyles DS, Schnell MJ. Interferon response and viral evasion by members of the family rhabdoviridae. *Viruses*. 2009; 1:832–851. [PubMed: 21994572]
- Finkelstein D, Werman A, Novick D, Barak S, Rubinstein M. LDL receptor and its family members serve as the cellular receptors for vesicular stomatitis virus. *Proc Natl Acad Sci*. 2013; 110:7306–7311. [PubMed: 23589850]
- Hankins RW, Nagata K, Kato A, Ishihama A. Mechanism of influenza virus transcription inhibition by matrix (M1) protein. *Res Virol*. 1990; 141:305–314. [PubMed: 2392615]

- Hastie E, Cataldi M, Marriott I, Grdzlishvili VZ. Understanding and altering cell tropism of vesicular stomatitis virus. *Virus Res.* 2013; 176:16–32. [PubMed: 23796410]
- Heinrich BS, Cureton DK, Rahmeh AA, Whelan SPJ. Protein expression redirects vesicular stomatitis virus RNA synthesis to cytoplasmic inclusions. *PLoS Pathog.* 2010; 6:e1000958. [PubMed: 20585632]
- Hensel SC, Rawlings JB, Yin J. Stochastic kinetic modeling of vesicular stomatitis virus intracellular growth. *Bull Math Biol.* 2009; 71:1671–1692. [PubMed: 19459014]
- Iverson LE, Rose JK. Localized attenuation and discontinuous synthesis during vesicular stomatitis virus transcription. *Cell.* 1981; 23:477–484. [PubMed: 6258804]
- Lichty BD, Power AT, Stojdl DF, Bell JC. Vesicular stomatitis virus: re-inventing the bullet. *Trends Mol Med.* 2004; 10:210–216. [PubMed: 15121047]
- Lim K, Lang T, Lam V, Yin J. Model-based design of growth-attenuated viruses. *PLoS Comput Biol.* 2006; 2:e116. [PubMed: 16948530]
- Lyles, DS., Rupprecht, CE. *Rhabdoviridae*. 5. Philadelphia, PA: Lippincott Williams & Wilkins; 2007. p. 1363–1408.
- Panda D, Das A, Dinh PX, Subramaniam S, Nayak D, Barrows NJ, Pearson JL, Thompson J, Kelly DL, Ladunga I, Pattnaik AK. RNAi screening reveals requirement for host cell secretory pathway in infection by diverse families of negative-strand RNA viruses. *Proc Natl Acad Sci U S A.* 2011; 108:19036–19041. [PubMed: 22065774]
- Rawlings, JB., Ekerdt, JG. *Chemical reactor analysis and design fundamentals*. Nob Hill Publishing, LLC; 2002.
- Rose JK. Complete intergenic and flanking gene sequences from the genome of vesicular stomatitis virus. *Cell.* 1980; 19:415–421. [PubMed: 6244108]
- Staeheli P, Pavlovic J. Inhibition of vesicular stomatitis virus mRNA synthesis by human MxA protein. *J Virol.* 1991; 65:4498–4501. [PubMed: 1649344]
- Stojdl DF, Lichty BD, TenOever BR, Paterson JM, Power AT, Knowles S, Marius R, Reynard J, Poliquin L, Atkins H, Brown EG, Durbin RK, Durbin JE, Hiscott J, Bell JC. VSV strains with defects in their ability to shutdown innate immunity are potent systemic anti-cancer agents. *Cancer Cell.* 2003; 4:263–275. [PubMed: 14585354]
- Suryanarayana K, Bacsko K, ter Meulen V, Wagner RR. Transcription inhibition and other properties of matrix proteins expressed by M genes cloned from measles viruses and diseased human brain tissue. *J Virol.* 1994; 68:1532–1543. [PubMed: 8107216]
- Thomas D, Newcomb WW, Brown JC, Wall JS, Hainfeld JF, Trus BL, Steven AC. Mass and molecular composition of vesicular stomatitis virus: A scanning transmission electron microscopy analysis. *J Virol.* 1985; 54:598–607. [PubMed: 2985822]
- Timm A, Yin J. Kinetics of virus production from single cells. *Virology.* 2012; 424:11–17. [PubMed: 2222212]
- Wertz GW, Perepelitsa VP, Ball LA. Gene rearrangement attenuates expression and lethality of a nonsegmented negative strand RNA virus. *Proc Natl Acad Sci U S A.* 1998; 95:3501–3506. [PubMed: 9520395]
- Whitaker-Dowling PA, Wilcox DK, Widnell CC, Youngner JS. Interferon-mediated inhibition of virus penetration. *Proc Natl Acad Sci.* 1983; 80:1083–1086. [PubMed: 6189119]
- Wu, CFJ., Hamada, MS. *Experiments: Planning, analysis, and optimization*. 2. Wiley; 2009.
- Zhu Y, Yongky A, Yin J. Growth of an RNA virus in single cells reveals a broad fitness distribution. *Virology.* 2009; 385:39–46. [PubMed: 19070881]

Figure 1. Simplified diagram of VSV transcription or replication. The virion deposits negative stranded genomic RNA and polymerase into the cell, which generates primary messenger RNA (plus-stranded) through transcription. After sufficient levels of new viral N protein accumulates, a fraction of the polymerase/genome complexes switch to replication activity. Replication proceeds through a full-length plus-stranded genomic RNA, which is the template for the full-length negative genomic RNA.

Figure 2. VSV N mRNA and genome kinetics in cycloheximide treated BHK cells. BHK cells were treated with 50 ug/mL cycloheximide. Medium and low MOI are 10 and 100-fold dilutions of high MOI samples, respectively. Samples were collected in duplicate as described in Methods. (A) mRNA and genome data from cycloheximide treated cells. (B) Model reactions and differential equation solutions. Parameter tables: k_t is the transcription constant (mRNA/genome/h), k_d is the genome degradation constant (h^{-1}), and IC is the initial condition (genomes).

Figure 3.

VSV N mRNA and genome kinetics in BHK cells. Medium and low MOI are 10 and 100-fold dilutions of high MOI samples, respectively. Samples were collected in duplicate as described in Methods. **(A)** mRNA and genome data from cycloheximide treated cells. **(B)** Model reactions and differential equation solutions. Parameter tables: k_t is the transcription constant (mRNA/genome/h), τ is the time delay before replication, k_r is the genome replication constant (h^{-1}), k_d is the genome degradation constant ($\text{genomes}^{-1}\text{h}^{-1}$), and IC is the initial condition (genomes).

Figure 4.

VSV N mRNA and genome kinetics in PC3 cells. Medium and low MOI are 10 and 100-fold dilutions of high MOI samples, respectively. Samples were collected in duplicate as described in Methods. **(A)** mRNA and genome data from cycloheximide treated cells. **(B)** mRNA and genome data from untreated cells. Parameter tables: k_t is the transcription constant (mRNA/genome/h), τ is the time delay before replication, k_r is the genome replication constant (h^{-1}), k_d is the genome degradation constant ($\text{genomes}^{-1}\text{h}^{-1}$), and IC is the initial condition (genomes). mRNA data during early infection for low MOI (Panel C) was below the qRT-PCR detection limit and omitted from the plot and analysis.

Figure 5.

VSV N mRNA and genome kinetics in IFN stimulated or untreated PC3 cells. PC3 cells were treated with 1,000 U/mL human IFN-beta 1 h before infection, and co-treated; or not treated. Medium and low MOI are 10 and 100-fold dilutions of high MOI samples, respectively. Samples were collected in duplicated as described in Methods. **(A)** N mRNA and genome measures and model for untreated PC3 cells. **(B)** N mRNA and genome measures for IFN treated PC3 cells. Parameter tables: k_t is the transcription constant (mRNA/genome/h), τ is the time delay before replication, k_r is the genome replication constant (h^{-1}), k_d is the genome degradation constant ($\text{genomes}^{-1}\text{h}^{-1}$), and IC is the initial condition (genomes).