JVI Journals.ASM.org

# A Vesiculovirus Showing a Steepened Transcription Gradient and Dominant *trans*-Repression of Virus Transcription

**Erin N. Hodges,[a] Bianca S. Heinrich,[b] and John H. Connor[a]**

Department of Microbiology, Boston University School of Medicine, Boston, Massachusetts, USA,[a] and Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, USA[b]

**Vesicular stomatitis virus (VSV) is a prototype nonsegmented, negative-sense virus used to examine viral functions of a broad family of viruses, including human pathogens. Here we demonstrate that $S_2$ VSV, an isolate with a small plaque phenotype compared to other Indiana strain viruses, has a transcription defect resulting in an altered pattern and rapid decline of transcription. The $S_2$ VSV transcription gradient is dominant over the wild-type transcription in a coinfection. This is the first characterization of an altered gradient of transcription not dependent on RNA template sequence or host response and may provide insight into new approaches to viral attenuation.**

Vesicular stomatitis virus (VSV) is a prototype nonsegmented, negative-strand (NNS) RNA virus with robust growth kinetics and is highly genetically tractable in laboratory conditions. Because of this, VSV has frequently been used to probe fundamental questions about virus replication. Specifically, the RNA transcription and replication strategy used by NNS viruses was first described and extensively characterized with VSV (1, 21, 24–26, 33).

VSV transcription requires the negative-sense RNA genome and three viral proteins. The large polymerase subunit (L) carries out the enzymatic functions of the polymerase, including transcription, capping, methylation, and polyadenylation (5, 23, 33). The phosphoprotein (P) is a required cofactor for the polymerase and interacts with both L and the nucleocapsid protein (N). N encapsidates the viral genome, allowing the RNA-dependent RNA polymerase (RdRp) to access the RNA and preventing degradation or detection by the host cell. The VSV polymerase complex consists of an oligomer of P for each molecule of L (8, 14, 15, 30). Transcription of viral mRNAs occurs in an obligate sequential stop-start manner beginning with the N gene near the 3′ end of the genome. Reinitiation of transcription between genes is not completely efficient, leading to a gradient of mRNA transcription along the genome. The mRNAs that are transcribed from regions closest to the 3′ promoter are transcribed in highest abundance, while genes further from the 3′ end are transcribed in lower abundance.

Here we show that an isolate of VSV previously demonstrated to be attenuated has a steeper gradient of transcription that is dominant over wild-type VSV. This suggests that the ability of the polymerase to reinitiate transcription is dependent not only on the *cis*-acting genomic sequence but also on the protein components of the polymerase complex.

$S_2$ VSV was originally isolated from an undifferentiated VSV-Indiana pool as a small plaque strain and clonally selected five times to ensure phenotype stability (39). This small plaque isolate was shown to be 1,000 times less lethal than wild-type virus in animal models but still able to induce a robust interferon response (39). Further characterization showed that $S_2$ VSV transcribes more small viral mRNA species (12S to 18S; N, P, M and G mRNA) but less of the largest viral RNA species (40S; replication products) than wild-type VSV (5, 12, 23, 32).

For our studies both wild-type VSV-San Juan and $S_2$ VSV were obtained and plaque purified and the growth attenuation earlier reported was verified. To quantify the observed attenuation, we carried out a time course of infection with wild-type and $S_2$ VSV and measured the titer of virus released into supernatant fluid by plaque assay on BHK-21 cells. At both low (0.1) and high (10) multiplicity of infection (MOI), $S_2$ VSV grows to a log lower titer than wild-type virus (Fig. 1A and B). The growth deficiency of $S_2$ VSV was also observed in Madin-Darby bovine kidney (MDBK) cells (data not shown).

In an attempt to define specific genetic differences between $S_2$ VSV and wild-type Indiana VSV, we sequenced the viral RNA from $S_2$ VSV-infected cells. Figure 1C shows a graphic representation of the VSV genome. Each line represents a nucleotide difference in the $S_2$ sequence from the published wild-type San Juan VSV sequence (GI 335873). Lines above the genome represent nucleotide changes that do not affect the predicted protein sequence. Lines below the genome represent nucleotide changes that result in predicted amino acid changes. Table 1 provides the number of nucleotide changes in each region of the $S_2$ genome, compared to wild-type Indiana strains San Juan (GI 335873) and Mudd-Summers (GI 194272583), as well as the number of predicted amino acid changes to each viral protein. The $S_2$ VSV genomic sequence shows significant differences from both wild-type VSV strains at both the nucleotide (314 and 303 differences, respectively) and protein (83 and 79 differences) levels. $S_2$ also shows significant differences from the $L_1$ strain of VSV that was isolated from the same nonclonal virus stock (39) (data not shown). As Fig. 1C indicates, these differences between $S_2$ and wild-type strains are not clustered to one region but are spread throughout all five viral proteins. Since $S_2$ VSV contains a large number of uncharacterized amino acid differences from known wild-type strains, it is difficult to predict which sequence differ-

**Received** 10 February 2012 **Accepted** 28 May 2012
**Published ahead of print** 6 June 2012
Address correspondence to John H. Connor, jhconnor@bu.edu.

doi:10.1128/JVI.00358-12

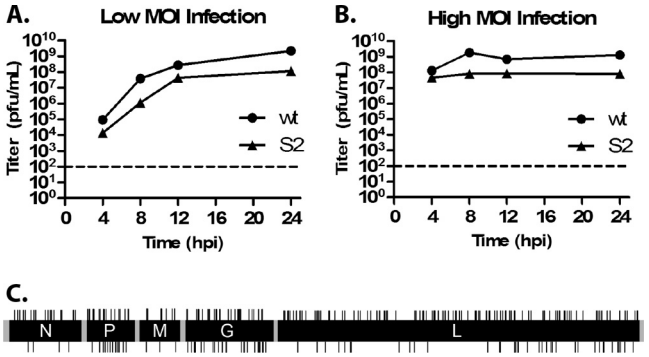


**FIG 1** Growth and sequence of $S_2$ VSV. (A) Growth curves of wild-type and $S_2$ VSV at an MOI of 0.01 in BHK-21 cells. Wild-type VSV shown by closed circles; $S_2$ VSV by closed triangles. Dashed line indicates the minimum detection level of the assay. (B) Growth curves of wild-type and $S_2$ VSV at an MOI of 10 in BHK-21 cells. Wild-type VSV shown by closed circles; $S_2$ VSV by closed triangles. Data shown are the averages of results from three experiments with standard errors (error bars not visible at this scale). (C) Graphic representation of the sequence differences between $S_2$ VSV and the published VSV-San Juan sequence (GI 61833) in coding regions of the genome. Coding regions are represented as solid boxes; noncoding regions are in gray. Single-letter abbreviations are used to denote proteins encoded. Each line above the genome represents a silent mutation; each line below the genome represents a nucleotide change that results in an amino acid change.

ence is responsible for the attenuation phenotype. For this reason, we decided to determine where in the virus life cycle $S_2$ VSV was defective.

Viral RNA production was examined by $^3$H-uridine incorporation to visualize the production of individual mRNAs. Cells mock infected or infected with wild-type or $S_2$ VSV at an MOI of 10 for increasing times were labeled with 10 μCi/ml $^3$H-uridine (Perkin-Elmer) for 2 h in the presence of 2 μg/ml actinomycin D to block host transcription. Total RNA from the cells was separated on a 1% agarose-formaldehyde gel to visualize individual mRNAs (Fig. 2A). Mock-infected cells show no transcription in the presence of actinomycin D, as expected (lanes 1 and 2). Wild-type VSV at 4 h postinfection (hpi) shows robust transcription of all of the viral mRNAs (lane 3). $S_2$ VSV also transcribes all of the viral mRNAs at 4 hpi (lane 4); however, the amount of each RNA species varies from that of the wild type. $S_2$ VSV makes more N mRNA at 4 hpi than wild-type VSV but less of each of the subsequent transcripts.

Calculation of the molar ratio of transcription (see reference 31) at 4 hpi demonstrates that the altered levels of mRNA production are the result of a steepened gradient of transcription (Fig. 2B). Wild-type VSV transcribes mRNA in ratios similar to those in previously published reports (20, 31), with an approximately 30% reduction in transcription after each gene junction. In contrast, $S_2$ VSV transcription decreases more quickly, as can be seen from the steeper slope, demonstrating an enhanced gradient of transcription. This difference in relative amounts of transcription can be seen at each position in the genome. For example, while wild-type VSV transcribes 40.4% of the levels of G mRNA as it transcribes of N mRNA, $S_2$ VSV transcribes only 19.4% of G, relative to N mRNA levels. We calculate that the decrease in transcription at each gene junction is approximately 50% in $S_2$ VSV transcription. Total viral RNA production shows that both viruses transcribe similar total amounts of RNA at 4 hpi (Fig. 2C), which is consistent with previous descriptions of $S_2$ VSV (12). At later time points in infection, wild-type VSV transcription increases (Fig. 2C, lanes 5, 7, and 9) while $S_2$ VSV peaks at the early time point and decreases later (Fig. 2C, lanes 6, 8, and 10).

To verify that the increase in N mRNA transcription is not a result of leader-N read-through, we examined the accumulation of RNA containing the anti-genomic-sense VSV leader sequence. Total RNA from mock-infected cells or cells infected with wild-type or $S_2$ VSV was separated on a denaturing formaldehyde gel and probed with an oligonucleotide probe specific for the antigenomic leader sequence. As Fig. 2D shows, the only detectable RNA product that contains leader sequence is the full-length antigenome, found in cells infected with both wild-type and $S_2$ VSV at later time points (6 to 8 hpi).

The decreased transcription of mRNA by $S_2$ VSV suggests that viral messages would accumulate to lower levels in $S_2$-infected cells. We determined the total levels of mRNA in cells infected with wild-type or $S_2$ VSV at increasing times during infection by Northern blotting. Levels of total RNA were determined by probing for individual mRNAs with double-stranded DNA (dsDNA) gene-specific radiolabeled probes (Fig. 2E). No detectable mRNA was seen at 2 hpi, but by 4 hpi, all five mRNA species were able to be detected in wild-type VSV-infected cells. Wild-type VSV accumulation of all mRNA species stays at levels higher than $S_2$ at all later times in infection, even at this early time point when $S_2$ transcription is highest. These data are consistent with the conclusion that $S_2$ transcribes less mRNA than the wild type.

Although we would predict from Fig. 2A that N mRNA should accumulate to higher levels in $S_2$ VSV-infected cells than in wild-type-infected cells at 4 hpi, Fig. 2E shows more N mRNA in cells infected with wild-type VSV, even at this early time point. Since

**TABLE 1** $S_2$ VSV sequence comparison

| Gene | Region[a] | San Juan | | Mudd-Summers | |
|---|---|---|---|---|---|
| | | Nucleotide changes | Amino acid changes | Nucleotide changes | Amino acid changes |
| N | 5′ UTR | 0 | | 0 | |
| | Coding | 22 | 4 | 29 | 6 |
| | 3′ UTR | 0 | | 0 | |
| | IG | 0 | | 0 | |
| P | 5′ UTR | 0 | | 0 | |
| | Coding | 37 | 14 | 29 | 13 |
| | 3′ UTR | 0 | | 0 | |
| | IG | 0 | | 0 | |
| M | 5′ UTR | 1 | | 0 | |
| | Coding | 10 | 3 | 11 | 1 |
| | 3′ UTR | 5 | | 6 | |
| | IG | 0 | | 0 | |
| G | 5′ UTR | 2 | | 1 | |
| | Coding | 61 | 25 | 55 | 24 |
| | 3′ UTR | 16 | | 11 | |
| | IG | 0 | | 0 | |
| L | 5′ UTR | 0 | | 0 | |
| | Coding | 160 | 37 | 161 | 35 |
| | 3′ UTR | 0 | | 0 | |
| Total | | 314 | 83 | 303 | 79 |

[a]UTR, untranslated region; IG, intergenic region.

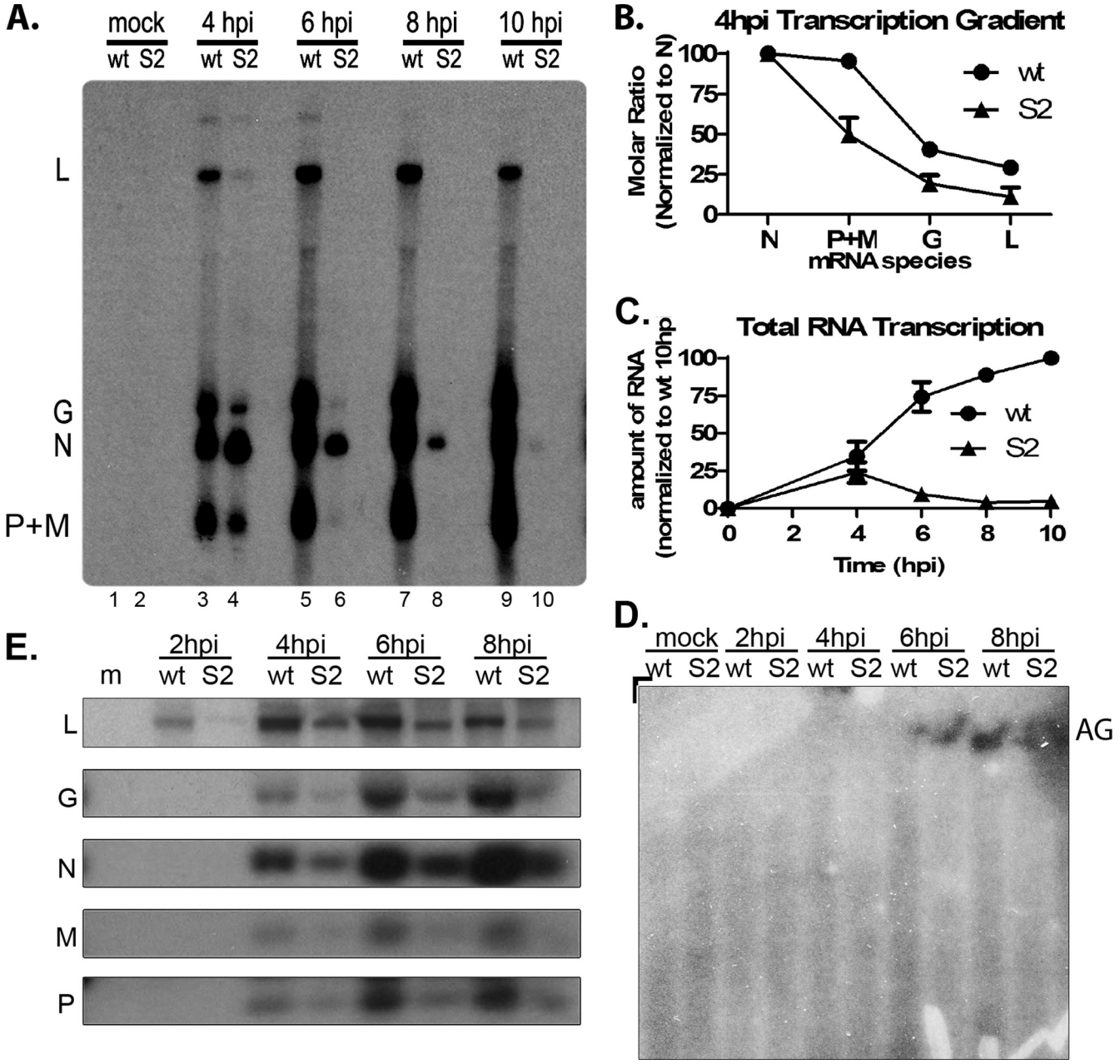


**FIG 2** RNA production in wild-type and $S_2$ VSV infected cells. (A) BHK cells infected with wild-type or $S_2$ VSV were labeled for 2 h with $^3$H-uridine in the presence of actinomycin D for the last 2 h of infection at times indicated. RNA from infected cells was separated on a denaturing formaldehyde gel. An autoradiograph of a representative experiment is shown. (B) Calculated molar ratios of wild-type and $S_2$ mRNA transcription products at 4 hpi. Wild-type VSV shown by closed circles; $S_2$ VSV by closed triangles. The data are averages of results from three separate experiments ± standard errors. (C) Total RNA production levels by wild-type and $S_2$ VSV from 4 to 10 hpi. Wild-type VSV shown by closed circles; $S_2$ VSV by closed triangles. The data are averages of three separate experiments ± standard errors. (D) Total RNA from BHK-21 cells mock infected (M) or infected with wild-type or $S_2$ VSV was separated on a denaturing formaldehyde gel, transferred to positively charged nylon, and probed using an oligonucleotide probe specific for the viral leader sequence. AG, antigenome. (E) Total RNA, as for panel D, was probed using dsDNA probes specific for each viral gene. A representative experiment is shown.

Fig. 2A shows the production of mRNA over a 2-h time period, it is possible that $S_2$ VSV transcription begins to decline prior to 4 hpi but the early overproduction masks this decreased production at later times during the labeling period.

$S_2$ VSV is the first described naturally occurring virus mutant that shows an inherent transcription gradient defect. Other reports have demonstrated that the ability of the VSV polymerase to transcribe a downstream gene is dependent on the length and sequence of intergenic regions (2, 3, 19, 29), a highly conserved two-nucleotide region between gene ends. As shown in Table 1, sequencing of the $S_2$ VSV genome has not revealed any mutations in these viral intergenic regions.

Having demonstrated that $S_2$ VSV was deficient in mRNA transcription, we asked whether the transcription deficiency could

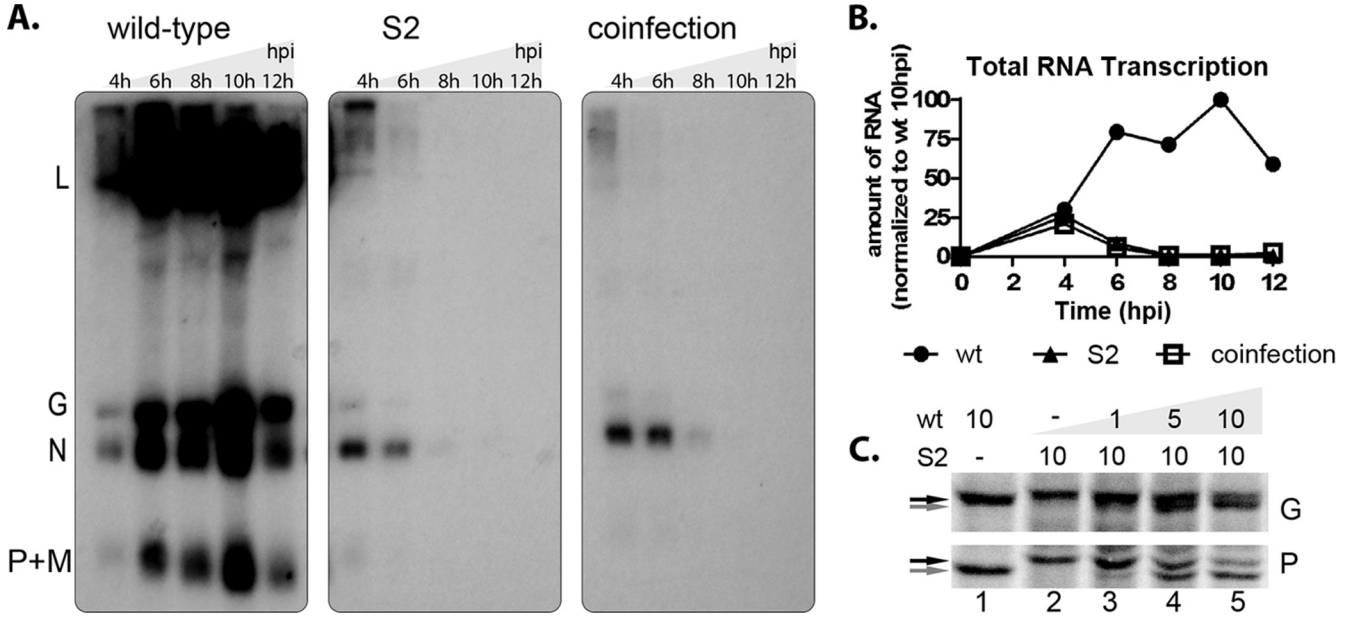


**FIG 3** RNA production in singly infected or coinfected cells. (A) BHK cells infected at an MOI of 10 with wild-type, $S_2$, or both (total MOI of 20) viruses were labeled with $^3$H-uridine for 2 h at increasing times during infection and analyzed as described above. (B) Total RNA production levels in infected cells from 4 to 12 hpi. Wild-type VSV shown by closed circles; $S_2$ VSV by closed triangles. Coinfection indicated by open squares. (C) BHK cells were infected with wild-type VSV, $S_2$ VSV, or both at the MOI listed above each lane. At 8 hpi, cells were labeled with [$^{35}$S]methionine/cysteine for 15 min and then lysed. Total protein was separated on an SDS-PAGE gel. A representative autoradiograph is shown. The gray arrow indicates the migration of wild-type proteins; the black arrow indicates the migration of $S_2$ proteins.

be complemented by wild-type virus. Cells were infected with wild-type or $S_2$ VSV or were coinfected with equal amounts of the two viruses. RNA production in these cells was labeled with $^3$H-uridine in the presence of actinomycin D as for Fig. 2A. The patterns of transcription of wild-type and $S_2$ VSV are the same as those seen in Fig. 2 (Fig. 3A, left and middle panels). The right panel of Fig. 3 shows the transcriptional activity in cells coinfected with both wild-type and $S_2$ VSV. The transcription profile of these cells identically replicates transcription in cells infected with $S_2$ VSV only (Fig. 3B).

To verify that both wild-type and $S_2$ VSV gene products were being expressed in coinfected cells, we took advantage of the fact that two of the $S_2$ viral products, the proteins P and G, migrate more slowly through an SDS-PAGE gel than wild-type proteins. Cells were infected with wild-type VSV alone, $S_2$ VSV alone, or $S_2$ with an increasing amount of wild-type VSV. At 8 hpi, total protein synthesis of these infected cells was labeled by incorporation of radioactive amino acids and separated by SDS-PAGE. When cells are coinfected with both viruses, both protein species are seen (lane 5), demonstrating that the gene products of both viruses are made in equimolar amounts (Fig. 3C). This argues that the lower level of transcription seen in Fig. 3A is an effect on both genomes.

These data indicate that $S_2$ VSV is capable of exerting *trans*-dominant control over wild-type virus transcription in the coinfected cells as a result of two possible mechanisms: the first possibility is that $S_2$ VSV produces a factor, likely a viral protein, which is dominant over the wild-type factor, and the second possibility is that $S_2$ VSV triggers a host cell response that is able to shut down viral transcription. To determine whether the $S_2$ transcription phenotype is dependent on the host cellular response, we performed *in vitro* transcription reactions in the absence of any possible cellular response.

Purified virions were detergent activated and incubated with [$\alpha$-$^{32}$P]GTP in a standard *in vitro* transcription reaction for 5 h (17). Transcription products from the first four genes of the VSV genome (N, M+P, and G) were able to be detected from both viruses (Fig. 4A). Calculation of the molar ratio shows that the $S_2$ transcription gradient *in vitro* is different than that of the wild type, as can be seen from the different slopes of the molar ratio curves in Fig. 4B. While the molar ratios *in vitro* differ from those previously seen in infected cells, these data are consistent with

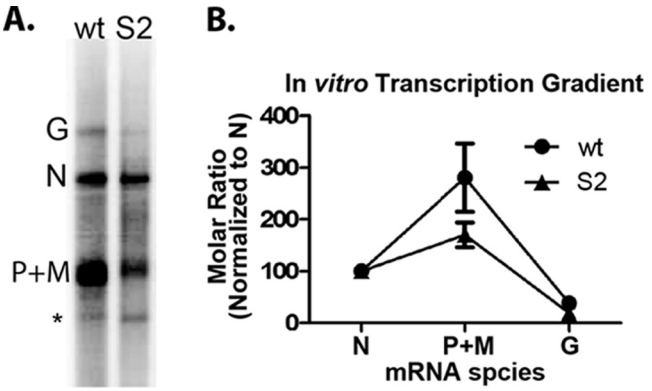


**FIG 4** RNA production by wild-type and $S_2$ VSV in a cell-free system. (A) *In vitro* transcription reactions were carried out using detergent-activated wild-type or $S_2$ virions, using [$^{32}$P]GTP as a trace label. RNA was purified and separated on an agarose-urea gel and exposed to a phosphorimager screen. Data shown are representative of three independent experiments. Asterisk indicates a background band seen in all reactions. (B) Calculated molar ratios of wild-type and $S_2$ mRNA transcription products. Wild-type VSV shown by closed circles; $S_2$ VSV by closed triangles. Data are averages of results from three separate experiments ± standard errors.

other published descriptions of *in vitro* transcription (28). These data duplicate the $S_2$ transcription deficiency seen in infected cells, showing that the $S_2$ defect in transcription is due to a defective viral signal, not the induction of a host factor. Previous investigation of altered transcription gradients in the related viruses human parainfluenza virus 5 (hPIV5) and measles virus have been shown to be dependent on the host immune response (6, 7).

We hypothesize that a component (or multiple components) of the $S_2$ viral polymerase complex contains a mutation that decreases its ability to reinitiate transcription at each gene junction. This process appears to be able to alter transcription from both wild-type and $S_2$ genomes in *trans* during a coinfection. The obligate sequential mechanism of transcription by VSV predicts that this would result in a greater percentage of the polymerase "falling off" the genome at each gene junction, leading to relatively fewer copies of each gene downstream and freeing the polymerase complex to be able to reinitiate transcription of the N mRNA at the 3′ end of the genome. *trans*-dominant mutant proteins are typically able to block wild-type viral function by multimerization in a complex with wild-type proteins that render the entire complex defective (reviewed in reference 18). Polymerase component mutants have previously been demonstrated to be dominant over wild-type virus (36–38). It is likely, given the large number of unique mutations found in the $S_2$ VSV sequence, that multiple mutations over more than one protein have occurred to stabilize this unique phenotype of the $S_2$ VSV polymerase.

Many of the NNS viruses are human pathogens with no available treatments or vaccines. Live-virus vaccines are able to induce a robust protective immune response; however, the use of live, attenuated mutants of highly pathogenic viruses would leave open the possibility of compensatory mutations restoring pathogenicity to the vaccine strain. VSV has been developed as a platform to present the pathogenic viral glycoproteins to the immune system in order to induce a protective immune response without exposure to a potentially deadly pathogen. VSV vectors expressing foreign glycoproteins have been demonstrated to be effective potential vaccines for many current and emerging human pathogens, including the hemorrhagic viruses Ebola and Marburg (13, 16), HIV (10), influenza virus (27), and hepatitis B virus (9). VSV is not a human pathogen, and its low seroprevalence among humans makes it an excellent vector candidate. However, as with other live, replicating viruses, some concerns remain about pathogenicity. Dominant negative mutants are ideal for vaccine development because they are able to induce a strong antiviral response, are restricted for growth *in vivo*, and are able to block the spread of revertants (11). The currently available seasonal flu vaccine FluMist uses the cold-adapted influenza virus dominant negative polymerase mutant and has been demonstrated to be a safe, effective vaccine (4, 22, 34, 35).

We have demonstrated that $S_2$ VSV is a dominant negative, attenuated mutant of VSV that is able to block wild-type viral transcription in *trans*. Use of $S_2$ VSV as a viral vector would take advantage of an attenuated virus and also reduce the chances of the spread of a pathogenic mutant. $S_2$ VSV has been shown to induce a robust interferon response but to be nonpathogenic in mice (39; unpublished data) and therefore is a good candidate for *in vivo* use. In addition, functional studies of $S_2$ VSV polymerase complex function could aid in the understanding of broad mechanisms of transcription by the NNS virus polymerases.

## ACKNOWLEDGMENTS

The authors thank Gail Wertz for her kind gift of the $S_2$ and $L_1$ viruses.

This work was supported by grant AI1096159 to J.H.C.

## REFERENCES

1. **Baltimore D, Huang AS, Stampfer M.** 1970. Ribonucleic acid synthesis of vesicular stomatitis virus. II. An RNA polymerase in the virion. Proc. Natl. Acad. Sci. U. S. A. **66**:572–576.
2. **Barr JN, Tang X, Hinzman E, Shen R, Wertz GW.** 2008. The VSV polymerase can initiate at mRNA start sites located either up or downstream of a transcription termination signal but size of the intervening intergenic region affects efficiency of initiation. Virology **374**:361–370.
3. **Barr JN, Whelan SP, Wertz GW.** 1997. Role of the intergenic dinucleotide in vesicular stomatitis virus RNA transcription. J. Virol. **71**:1794–1801.
4. **Belshe RB, Gruber WC.** 2001. Safety, efficacy and effectiveness of cold-adapted, live, attenuated, trivalent, intranasal influenza vaccine in adults and children. Philos. Trans. R. Soc. Lond. B Biol. Sci. **356**:1947–1951.
5. **Both GW, Moyer SA, Banerjee AK.** 1975. Translation and identification of the mRNA species synthesized in vitro by the virion-associated RNA polymerase of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U. S. A. **72**:274–278.
6. **Carlos TS, Fearns R, Randall RE.** 2005. Interferon-induced alterations in the pattern of parainfluenza virus 5 transcription and protein synthesis and the induction of virus inclusion bodies. J. Virol. **79**:14112–14121.
7. **Cattaneo R, Rebmann G, Baczko K, ter Meulen V, Billeter MA.** 1987. Altered ratios of measles virus transcripts in diseased human brains. Virology **160**:523–526.
8. **Chen M, Ogino T, Banerjee AK.** 2006. Mapping and functional role of the self-association domain of vesicular stomatitis virus phosphoprotein. J. Virol. **80**:9511–9518.
9. **Cobleigh MA, Buonocore L, Uprichard SL, Rose JK, Robek MD.** 2010. A vesicular stomatitis virus-based hepatitis B virus vaccine vector provides protection against challenge in a single dose. J. Virol. **84**:7513–7522.
10. **Cooper D, et al.** 2008. Attenuation of recombinant vesicular stomatitis virus-human immunodeficiency virus type 1 vaccine vectors by gene translocations and G gene truncation reduces neurovirulence and enhances immunogenicity in mice. J. Virol. **82**:207–219.
11. **Crowder S, Kirkegaard K.** 2005. Trans-dominant inhibition of RNA viral replication can slow growth of drug-resistant viruses. Nat. Genet. **37**:701–709.
12. **Davis NL, Wertz GW.** 1980. A VSV mutant synthesizes a large excess of functional mRNA but produces less viral protein than its wild-type parent. Virology **103**:21–36.
13. **Falzarano D, Geisbert TW, Feldmann H.** 2011. Progress in filovirus vaccine development: evaluating the potential for clinical use. Expert Rev. Vaccines **10**:63–77.
14. **Gao Y, Greenfield NJ, Cleverley DZ, Lenard J.** 1996. The transcriptional form of the phosphoprotein of vesicular stomatitis virus is a trimer: structure and stability. Biochemistry **35**:14569–14573.
15. **Gao Y, Lenard J.** 1995. Cooperative binding of multimeric phosphoprotein (P) of vesicular stomatitis virus to polymerase (L) and template: pathways of assembly. J. Virol. **69**:7718–7723.
16. **Geisbert TW, et al.** 2009. Single-injection vaccine protects nonhuman primates against infection with Marburg virus and three species of Ebola virus. J. Virol. **83**:7296–7304.
17. **Heinrich BS, Cureton DK, Rahmeh AA, Whelan SP.** 2010. Protein expression redirects vesicular stomatitis virus RNA synthesis to cytoplasmic inclusions. PLoS Pathog. **6**:e1000958. doi:10.1371/journal.ppat.1000958.
18. **Herskowitz I.** 1987. Functional inactivation of genes by dominant negative mutations. Nature **329**:219–222.
19. **Hinzman EE, Barr JN, Wertz GW.** 2008. Selection for gene junction sequences important for VSV transcription. Virology **380**:379–387.
20. **Iverson LE, Rose JK.** 1981. Localized attenuation and discontinuous synthesis during vesicular stomatitis virus transcription. Cell **23**:477–484.
21. **Li T, Pattnaik AK.** 1999. Overlapping signals for transcription and replication at the 3′ terminus of the vesicular stomatitis virus genome. J. Virol. **73**:444–452.
22. **Maloy ML, Whitaker-Dowling P, Youngner JS.** 1994. Dominance of cold-adapted influenza A virus over wild-type viruses is at the level of RNA synthesis. Virology **205**:44–50.

23. **Morrison T, Stampfer M, Baltimore D, Lodish HF.** 1974. Translation of vesicular stomatitis messenger RNA by extracts from mammalian and plant cells. J. Virol. **13**:62–72.
24. **Moyer SA, Abraham G, Adler R, Banerjee AK.** 1975. Methylated and blocked 5′ termini in vesicular stomatitis virus in vivo mRNAs. Cell **5**:59–67.
25. **Ogino T, Banerjee AK.** 2007. Unconventional mechanism of mRNA capping by the RNA-dependent RNA polymerase of vesicular stomatitis virus. Mol. Cell **25**:85–97.
26. **Rhodes DP, Moyer SA, Banerjee AK.** 1974. In vitro synthesis of methylated messenger RNA by the virion-associated RNA polymerase of vesicular stomatitis virus. Cell **3**:327–333.
27. **Schwartz JA, et al.** 2011. Vesicular stomatitis virus-based H5N1 avian influenza vaccines induce potent cross-clade neutralizing antibodies in rhesus macaques. J. Virol. **85**:4602–4605.
28. **Sleat DE, Banerjee AK.** 1993. Transcriptional activity and mutational analysis of recombinant vesicular stomatitis virus RNA polymerase. J. Virol. **67**:1334–1339.
29. **Stillman EA, Whitt MA.** 1997. Mutational analyses of the intergenic dinucleotide and the transcriptional start sequence of vesicular stomatitis virus (VSV) define sequences required for efficient termination and initiation of VSV transcripts. J. Virol. **71**:2127–2137.
30. **Takacs AM, Banerjee AK.** 1995. Efficient interaction of the vesicular stomatitis virus P protein with the L protein or the N protein in cells expressing the recombinant proteins. Virology **208**:821–826.
31. **Villarreal LP, Breindl M, Holland JJ.** 1976. Determination of molar ratios of vesicular stomatitis virus induced RNA species in BHK21 cells. Biochemistry **15**:1663–1667.
32. **Wertz GW, Levine M.** 1973. RNA synthesis by vesicular stomatitis virus and a small plaque mutant: effects of cycloheximide. J. Virol. **12**:253–264.
33. **Whelan SP, Barr JN, Wertz GW.** 2004. Transcription and replication of nonsegmented negative-strand RNA viruses. Curr. Top. Microbiol. Immunol. **283**:61–119.
34. **Whitaker-Dowling P, Lucas W, Youngner JS.** 1990. Cold-adapted vaccine strains of influenza A virus act as dominant negative mutants in mixed infections with wild-type influenza A virus. Virology **175**:358–364.
35. **Whitaker-Dowling P, Maassab HF, Youngner JS.** 1991. Dominant-negative mutants as antiviral agents: simultaneous infection with the cold-adapted live-virus vaccine for influenza A protects ferrets from disease produced by wild-type influenza A. J. Infect. Dis. **164**:1200–1202.
36. **Whitaker-Dowling P, Youngner JS.** 1987. Viral interference-dominance of mutant viruses over wild-type virus in mixed infections. Microbiol. Rev. **51**:179–191.
37. **Youngner JS, Dubovi EJ, Quagliana DO, Kelly M, Preble OT.** 1976. Role of temperature-sensitive mutants in persistent infections initiated with vesicular stomatitis virus. J. Virol. **19**:90–101.
38. **Youngner JS, Frielle DW, Whitaker-Dowling P.** 1986. Dominance of temperature-sensitive phenotypes. I. Studies of the mechanism of inhibition of the growth of wild-type vesicular stomatitis virus. Virology **155**:225–235.
39. **Youngner JS, Wertz G.** 1968. Interferon production in mice by vesicular stomatitis virus. J. Virol. **2**:1360–1361.