

# Simian varicella virus gene expression during acute and latent infection of rhesus macaques

Christine Meyer · Amelia Kerns · Alex Barron ·  
Craig Kreklywich · Daniel N. Streblow ·  
Ilhem Messaoudi

Received: 28 July 2011 / Revised: 26 September 2011 / Accepted: 6 October 2011 / Published online: 4 November 2011  
© Journal of NeuroVirology, Inc. 2011

**Abstract** Varicella zoster virus (VZV) is a neurotropic  $\alpha$ -herpesvirus that causes chickenpox during primary infection and establishes latency in sensory ganglia. Reactivation of VZV results in herpes zoster and other neurological complications. Our understanding of the VZV transcriptome during acute and latent infection in immune competent individuals remains incomplete. Infection of rhesus macaques with the homologous simian varicella virus (SVV) recapitulates the hallmarks of VZV infection. We therefore characterized the SVV transcriptome by quantitative real-time reverse transcriptase PCR during acute infection in bronchial alveolar

lavage (BAL) cells and peripheral blood mononuclear cells, and during latency in sensory ganglia obtained from the same rhesus macaques. During acute infection, all known SVV open reading frames (ORFs) were detected, and the most abundantly expressed ORFs are involved in virus replication and assembly such as the transcriptional activator ORF 63 and the structural proteins ORF 41 and ORF 49. In contrast, latent SVV gene expression is highly restricted. ORF 61, a viral transactivator and latency-associated transcript, is the most prevalent transcript detected in sensory ganglia. We also detected ORFs A, B, 4, 10, 63, 64, 65, 66, and 68 though significantly less frequently than ORF 61. This comprehensive analysis has revealed genes that potentially play a role in the establishment and/or maintenance of SVV latency.

**Electronic supplementary material** The online version of this article (doi:10.1007/s13365-011-0057-y) contains supplementary material, which is available to authorized users.

C. Meyer · A. Kerns · A. Barron · D. N. Streblow · I. Messaoudi  
Vaccine and Gene Therapy Institute,  
Oregon Health and Science University,  
Beaverton, USA

C. Meyer · C. Kreklywich · D. N. Streblow · I. Messaoudi  
Department of Molecular Microbiology and Immunology,  
Oregon Health and Science University,  
Portland, USA

I. Messaoudi  
Division of Pathobiology and Immunology,  
Oregon National Primate Research Center,  
Beaverton, USA

C. Kreklywich  
Department of Surgery, Oregon Health and Science University,  
Portland, USA

I. Messaoudi (✉)  
Vaccine and Gene Therapy Institute,  
Oregon National Primate Research Center,  
505 NW 185th Avenue,  
Beaverton, OR 97006, USA  
e-mail: messaoud@ohsu.edu

**Keywords** Herpesvirus · Simian varicella virus · Gene expression · Latency · Rhesus macaque

## Introduction

Primary infection with varicella zoster virus (VZV), a neurotropic  $\alpha$ -herpesvirus, results in chickenpox (varicella). VZV then establishes a lifelong latent infection in sensory ganglia such as the trigeminal and dorsal root ganglia. Reactivation of VZV leads to herpes zoster (shingles), which causes significant morbidity and occasionally mortality in aged and immunocompromised individuals (Cohen et al. 2007). The currently approved vaccine Zostavax<sup>®</sup> reduces the incidence of shingles by 51% (Oxman et al. 2005), leaving a significant portion of the vaccine recipients still susceptible to VZV reactivation. To improve vaccine efficacy and develop novel therapeutics, we need to increase our understanding of the viral factors that control VZV latency and reactivation. One approach is to characterize

the viral transcriptome, which has the potential to identify viral genes that can be used as subunit vaccine components, targets for novel therapeutics, or to generate rationally attenuated vaccine strains.

To identify viral genes that impact VZV acute infection, latency, and reactivation, several studies characterized differences in transcriptional levels of VZV open reading frames (ORFs) between lytic and latent infection. Although VZV acute transcription has not been investigated in the natural host, all 68 unique VZV ORFs are expressed in virus-infected cells in culture (Cohrs et al. 2003b; Kennedy et al. 2005; Nagel et al. 2009). VZV transcription in latently infected human sensory ganglia has been examined by various methods including Northern blot hybridization, cDNA libraries, in situ hybridization (ISH), reverse transcriptase PCR (RT-PCR), and quantitative and multiplex PCR. The core transcripts detected in these studies include ORFs 4, 21, 29, 62, 63, and 66 (Cohrs et al. 1995, 1996, 2000, 2003a; Cohrs and Gilden 2007; Kennedy et al. 2000, 2001; Nagel et al. 2011). The results from these studies demonstrate that VZV latency is associated with the expression of a limited number of viral genes, and unlike other  $\alpha$ -herpesviruses, VZV is not known to encode latency-associated transcripts (LATs) (Cohen et al. 2007). One potential constraint of VZV transcriptional studies during latency is that the sensory ganglia examined are sometimes obtained from older individuals suffering from a number of co-morbidities such as cancer and sepsis, which could impact viral gene expression.

We developed a nonhuman primate (NHP) model where rhesus macaques (*Macaca mulatta*) are infected with the highly homologous simian varicella virus (SVV, *Cercopithecine herpesvirus 9*) (Messaoudi et al. 2009). The SVV and VZV genomes are collinear and share 70–75% DNA homology (Gray and Oakes 1984; Gray et al. 2001; Pumphrey and Gray 1992). Rhesus macaques infected with SVV display the hallmarks of VZV infection in humans: (1) appearance of generalized varicella, (2) development of cellular and humoral immunity with similar kinetics as those described in children during primary VZV infection, and (3) establishment of latency with limited transcriptional activity in sensory ganglia (Messaoudi et al. 2009). This model provides a unique opportunity to conduct a comprehensive analysis of the in vivo gene expression profiles during acute and latent infection in the same immune competent animal. Our analysis of the SVV transcriptome revealed that during acute infection, all known SVV ORFs were expressed to detectable levels, although the relative amount was specific for each gene. We also found that latency is associated with a limited transcriptional profile and that the expression of SVV ORF 61 is the most prevalent. Identifying the viral genes expressed from different tissues at different times during infection will aid in the selection of immunological targets

for vaccine strategies or therapeutic targets for drug development against VZV.

## Materials and methods

**Ethics statement** All animals were housed at the Oregon National Primate Research Center and were handled in accordance with good animal practices as defined by all relevant national and/or local animal welfare bodies. Animal work was approved by the Oregon National Primate Research Center Institutional Animal Care and Use Committee (IACUC#0779).

**Animals and samples** Rhesus macaques were infected intrabronchially with  $4 \times 10^5$  plaque-forming units (PFU) as previously described (Messaoudi et al. 2009). Peripheral blood mononuclear cells (PBMC), bronchial alveolar lavage cells (BAL), and sensory ganglia were collected from five juvenile (ages 3–5 years old) rhesus macaques (RM, *Macaca mulatta*) as previously described (Messaoudi et al. 2009). One million BAL cells were collected on 3 and 7 days post-infection (dpi), and one million PBMC cells were collected on 10 dpi. Cell samples were treated with Trizol (Invitrogen, Carlsbad, CA) and stored at  $-80^\circ\text{C}$  until further processing. Animals were euthanized at  $>72$  days post-infection. Sensory ganglia: trigeminal (TG), cervical, thoracic, and lumbar–sacral dorsal root ganglia (DRG-C, DRG-T, and DRG-L/S, respectively) were collected in Trizol and stored at  $-80^\circ\text{C}$  until analysis. SVV viral loads in whole blood (WB), BAL, and sensory ganglia were measured by quantitative real-time PCR (qPCR) using primers and probe specific for SVV ORF 21 as previously described (Messaoudi et al. 2009) (Supplementary Table 1).

**RNA isolation and amplification** Total RNA was isolated from acutely infected PBMC and BAL cells and latently infected sensory ganglia from infected rhesus macaques using the Trizol method according to the manufacturer's protocol. First, strand cDNA was synthesized from 1  $\mu\text{g}$  of total RNA using 0.5  $\mu\text{M}$  T7 oligo(dT)-T7 primer and 250 U SuperScript III reverse transcriptase (Invitrogen). Double-stranded cDNA was generated by the addition of second-strand buffer (Invitrogen) as per manufacturer's instruction. Double-stranded cDNA was purified using phenol-chloroform-isoamyl alcohol extraction and concentrated in Amicon Ultra centrifugal filters (Millipore, Billerica, MA) before being amplified using the T7 MEGAscript Kit (Ambion, Austin, TX) according to the manufacturer's method. The amplified RNA (aRNA) was purified using the RNeasy Mini Kit (Qiagen, Valencia, CA) using the manufacturer's protocol. Five micrograms of aRNA was reverse transcribed with 350 U SuperScript III reverse transcriptase in

the presence of 9 µg of random primers (Invitrogen). Double-stranded cDNA and second-round aRNA were generated as described above and repeated for a total of three rounds of RNA amplification.

**Quantitative real-time reverse transcriptase PCR detection of SVV gene expression** Quantitative real-time reverse transcriptase PCR (RT-qPCR) was used to quantify SVV gene expression. Single-stranded cDNA was generated from third-round aRNA using the High Capacity Reverse Transcription Kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. The cDNA was analyzed by RT-qPCR using primer and probe sets specific for each ORF in the SVV genome designed using Primer Express software (Applied Biosystems). A list of the primer and probe sequences used for this study are presented in Supplementary Table 1. RT-qPCR was performed using specific TaqMan probes and 2× Gene Expression Master Mix (Applied Biosystems) or Maxima Probe/ROX qPCR Master Mix (2×) (Fermentas, Glen Burnie, MD). Following an initial 10-min 95°C step, 40 cycles of 15 s at 95°C and 1 min at 60°C were completed using a StepOnePlus RT-qPCR machine (Applied Biosystems). Plasmids containing each target amplicon or synthesized amplicons were used as quantification standards. A positive signal in our analysis required that any given SVV gene express at least 100 copies in 1 µg of total RNA. Copy numbers are reported as the average of triplicate RT-qPCR analyses for each sample and are within 25% standard deviation for the population. The reported average copy numbers are not absolute but relative to our sample set.

## Results

**SVV infection and viremia** Five juvenile RM were infected intrabronchially with  $4 \times 10^5$  PFU. All RMs displayed the

hallmarks of SVV infection including the development of varicella and viremia that resolved approximately 21 dpi. All RMs developed varicella by 10 dpi, and a qualitative assessment of rash for each animal during acute SVV infection showed that the severity varied between animals with RM 25043 displaying the greatest number of skin lesions followed by RM 25339, RM 20226 and RM 24953, and lastly RM 24943 (Table 1). SVV establishes latency within the sensory ganglia, and as early as 2 months following varicella, SVV DNA can be found exclusively in ganglia and not in other tissues (Grinfeld and Kennedy 2007; Mahalingam et al. 1991; Messaoudi et al. 2009; Ou et al. 2007). Thus, our SVV latency analysis was investigated in sensory ganglia collected greater than 72 dpi. To follow the SVV viral load during acute infection, we analyzed both whole blood and BAL samples. DNA was extracted from a portion of both whole blood and BAL cells collected from each RM to measure viral genome levels by qPCR using a primer and probe set specific for SVV ORF 21. Table 1 shows the genome copies per microgram of DNA in BAL at 3 and 7 dpi and in whole blood at 10 dpi. Peak SVV DNA levels are usually detected in BAL between 3 and 7 dpi and in whole blood between 7 and 10 dpi. However, a sufficient number of PBMCs to complete the transcriptional analysis were only available at 10 dpi. Viral DNA loads were significantly higher and peaked earlier during acute infection ( $10^2$ – $10^5$  copies/µg) in BAL than in whole blood (10–100 copies/µg), likely reflecting the route of infection. SVV viral loads in the BAL correlated with varicella severity during acute infection with RM 25043 and RM 25339 displaying the most extensive rash and the highest viral loads (Table 1). To assess the latent viral burden in the same SVV-infected RMs, a portion of the sensory ganglia collected at day 73 (RM 24953 and 25043), day 77 (RM 24943 and 25339), or day 178 (RM 20226) post-infection was used for SVV DNA analysis. Table 1 lists the genome copy number per microgram DNA for TG, DRG-C, DRG-T, and DRG-L/S.

**Table 1** Varicella severity and SVV DNA viral loads

Rash severity		Animal ID				
		RM 20226 ++	RM 24943 +	RM 24953 ++	RM 25043 ++++	RM 25339 +++
dpi	Sample	SVV genomic copies per µg DNA				
3	BAL	N/A	142	5,951	2,164,348	371,314
7	BAL	N/A	10,998	22,499	477,919	2,011,551
10	WB	N/A	20	N/D	80	103
>72	TG	448	90	164	N/D	47
	DRG-C	49	89	143	N/D	56
	DRG-T	101	79	175	60	51
	DRG-L/S	233	67	60	N/D	50

N/A sample not available, N/D viral DNA not detected, + approximately ten lesions

All of the sensory ganglia tested were positive for SVV DNA except in one animal (RM 25043) where one of four ganglia was positive for SVV DNA. The tissue sections used for analysis of SVV DNA viral loads were not the same tissue sections used for transcriptional analysis; consequently, RM 25043 does show SVV gene expression during latency in all sensory ganglia (Fig. 2a).

*SVV gene transcription profiles during acute infection in rhesus macaques* We monitored in vivo SVV gene expression in five infected RMs; the tissues available for transcriptional analysis during the acute phase of infection are listed in Table 2. In order to assess the entire SVV transcriptional profile for each sample and since we are only able to collect a limited number of cells from each animal, RNA was subjected to three rounds of amplification. Amplified RNA was reverse transcribed into cDNA and subjected to RT-qPCR using primer and probe sets specific for all 69 unique SVV ORFs (Supplementary Table 1). Before addressing the entire transcriptome for each sample, the total RNA was assessed by measuring the average copy number for the reference gene glutathione synthetase (GSS) by RT-qPCR. GSS levels were equivalent among the samples tested (data not shown). The RNA amplification process can produce bias in the results by over- or under-representing RNA transcripts compared to the starting material. Therefore, we compared the average copy number for a subset of SVV genes by RT-qPCR in the original and amplified sample, and the relative abundance did not differ significantly (ORFs 4, 21, and 61; data not shown). Our analysis does not include the poly(A) negative fraction of RNA.

To characterize the acute SVV gene expression profiles in vivo, we analyzed BAL cells at 3 and 7 dpi and PBMC at 10 dpi. Our results demonstrate that SVV gene transcription is evident as early as 3 dpi in the BAL with 71% of SVV ORFs expressed at detectable levels (Fig. 1a). The highest expressed SVV ORFs at 3 dpi in BAL include ORF 57, ORF 41, ORF 55, and ORF 63 representing viral proteins whose functions are unknown, structural, DNA synthesis, and transcriptional activation, respectively. The number and abundance of SVV genes expressed increases from day 3 to

day 7 post-infection in the BAL at which point transcripts associated with every SVV ORF are detected at different intensities between animals but with similar profiles (Fig. 1a–c). The viral ORFs highly expressed and in common to all three animals during acute infection at 7 dpi in BAL encode a transcriptional activator (ORF 63) and virion structural proteins (ORFs 49 and 41). Other ORFs highly expressed in two of three animals include ORF 23 (capsid protein), ORF 53 (gamma-1 protein, unknown), and ORF 65 (tegument phosphoprotein). Table 3 lists the relative copy numbers for all SVV ORFs in the three animals tested at 3 and 7 dpi.

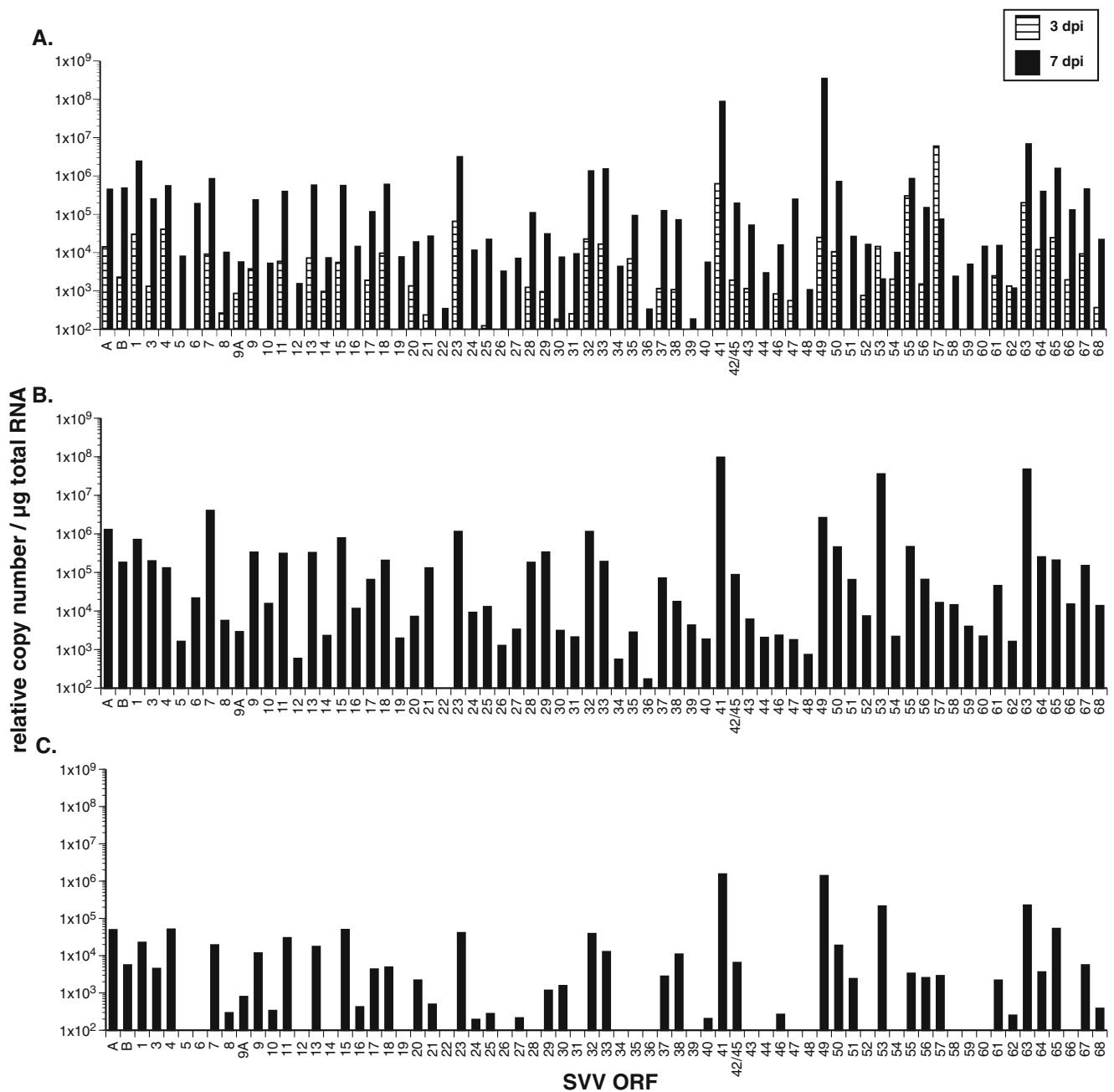
The transcriptional profile at 7 dpi in the BAL for RM 25339 (Fig. 1b) is comparable to RM 25043 (Fig. 1a). All SVV genes are detected with the exception of ORF 22, a tegument protein detected in RM 25043 at less than 400 copies per microgram of total RNA, which is not detected in RM 25339 (Table 3). RM 24953 (Fig. 1c) on the other hand, displays decreased viral gene transcription at 7 dpi in the BAL, both in abundance and the number of SVV viral ORFs detected. The transcriptional profile of RM 24953 at 7 dpi is reminiscent of RM 25043 at 3 dpi in the BAL. Interestingly, the viral load detected in the BAL at 7 dpi and severity of varicella at 10 dpi of RM 24953 were lower than those of RM 25043 or RM 25339 (Table 1) suggesting a quicker resolution of the acute SVV infection in this animal, which may have affected the levels of viral transcription in the BAL. In summary, with the exception of ORF 22, we were able to detect all known SVV genes during acute infection in the BAL at 7 dpi in at least two of three rhesus macaques examined.

Our previous data suggested that SVV viral loads peaked in the blood at a later time post-infection than in the BAL (10 vs. 7 dpi), most likely due to the route of infection (intrabronchial); thus, we analyzed SVV gene expression in isolated PBMCs collected at 10 dpi for animals 20226, 24943, and 25339. In contrast to our observations in the BAL, only 18 SVV ORFs were detected in PBMCs at 10 dpi in the three animals tested. ORF 32 (phosphoprotein) and ORF 54 (viral DNA cleavage/packaging) were detected in all three PBMC samples (Table 4). In agreement with acute infection in the BAL, ORFs 23, 41, and 63 were amongst the

**Table 2** Animal tissues evaluated for SVV transcriptome

Animal ID	Acute		Latent				
	BAL	PBMC	dpi	TG	DRG-C	DRG-T	DRG-L/S
RM 20226		10 dpi	178	+	+	+	+
RM 24943		10 dpi	77	+			+
RM 24953	7 dpi		73		+	+	+
RM 25043	3 and 7 dpi		73	+	+	+	+
RM 25339	7 dpi	10 dpi	77	+		+	+

⊕ Indicates that the tissue was evaluated for the SVV transcriptome



**Fig. 1** SVV transcriptome in BAL from acutely infected animals. Total RNA was isolated from  $1 \times 10^6$  BAL cells. RNA was amplified and analyzed by RT-qPCR using primer and probe sets specific for

each viral ORF. Data shown are the average copy number for triplicate reactions per sample. **a** RM 25043 (3 (lined) and 7 dpi (black)), **b** RM 25339 7 dpi (black), **c** RM 24953 7 dpi (black)

most highly expressed viral ORFs in PBMCs at 10 dpi in two of three animals.

*SVV gene transcription profiles during latent infection of rhesus macaques* Lastly, we investigated SVV gene expression during latency ( $>72$  dpi) in sensory ganglia (TG, DRG-C, DRG-T, and DRG-L/S) isolated from the same five animals described above. The latently infected sensory ganglia

available for transcriptional analysis for each RM are listed in Table 2. As predicted, SVV gene transcription is highly restricted during latency (Fig. 2). Only 12 SVV ORFs were detected in all 16 sensory ganglia tested, and the average copy number for any individual ORF did not exceed 4,000 copies per microgram of total RNA (Table 5). ORF 61 was the most prevalent transcript detected during latent infection, present in at least one sensory ganglion in all animals tested

**Table 3** Acute SVV gene expression in BAL

ORF	3 dpi	7 dpi			Putative function or function based on known VZV or HSV-1 homology <sup>a</sup>
	Animal ID				
	RM 25043	RM 25043	RM 25339	RM 24953	
A	14,088	443,745	49,015	1,286,901	Truncated homolog ORF4, unknown function
B	2,284	477,472	5,586	181,221	Homolog VZV ORF S/L: cytoplasmic viral protein, unknown function
1	30,000	2,414,229	22,473	708,613	Membrane protein
3	1,315	250,046	4,479	197,397	Virion assembly
4	40,405	548,791	50,842	130,030	Transcriptional activator, IE2
5	0	7,977	0	1,611	Glycoprotein K
6	0	187,663	0	21,501	Component of DNA helicase–primase complex
7	9,043	838,375	19,217	4,006,752	Virion phosphoprotein
8	264	10,075	291	5,618	DeoxyUTPase
9A	863	5,631	792	2,876	Glycoprotein N
9	3,756	235,216	11,652	331,421	Tegument protein
10	0	5,209	334	15,474	Transcriptional activator, tegument protein
11	5,877	392,104	29,906	309,148	Tegument protein
12	0	1,531	0	583	Tegument protein
13	7,169	570,414	17,425	324,323	Thymidylate synthetase
14	961	7,243	0	2,288	Glycoprotein C
15	5,497	555,897	49,594	776,823	Membrane protein
16	0	14,380	420	11,518	Associated with DNA polymerase
17	1,888	115,013	4,321	64,863	Host shutoff virion protein
18	9,626	599,385	4,886	203,973	Ribonucleotide reductase, small subunit
19	0	7,681	0	1,955	Ribonucleotide reductase, large subunit
20	1,346	18,847	2,185	7,169	Capsid protein
21	236	26,683	493	129,625	Tegument protein
22	0	344	0	0	Tegument protein
23	65,188	3,150,427	40,813	1,142,928	Capsid protein
24	0	11,424	193	9,122	Membrane phosphoprotein
25	123	21,968	276	12,848	Viral DNA cleavage/packaging
26	0	3,234	0	1,262	DNA cleavage/packaging
27	0	7,018	212	3,319	Nuclear phosphoprotein
28	1,238	109,302	0	180,589	DNA polymerase
29	956	30,469	1,166	334,241	Single-stranded DNA binding protein
30	182	7,539	1,556	3,090	Viral DNA cleavage/packaging
31	254	9,219	0	2,096	Glycoprotein B
32	22,533	1,338,433	38,645	1,139,522	Phosphoprotein
33	16,667	1,518,226	12,678	190,107	Protease, capsid assembly protein
34	0	4,335	0	554	Viral DNA cleavage/packaging
35	6,832	91,870	0	2,800	Membrane protein
36	0	332	0	171	Thymidine kinase
37	1,148	122,694	2,781	70,765	Glycoprotein H
38	1,081	71,329	10,879	17,425	Virion protein
39	0	184	0	4,269	Envelope protein, viral egress
40	6	5,570	203	1,840	Major capsid protein
41	624,927	87,383,293	1,533,550	96,126,357	Capsid protein
42/45	1,905	192,439	6,478	86,900	Spliced product, viral terminase
43	1,148	51,669	0	6,073	Viral DNA cleavage/packaging

**Table 3** (continued)

ORF	3dpi		7dpi		Putative function or function based on known VZV or HSV-1 homology <sup>a</sup>
	Animal ID				
	RM 25043	RM 25043	RM 25339	RM 24953	
44	0	2,944	0	2,041	Virion protein
46	833	15,748	264	2,333	Tegument protein
47	556	245,474	0	1,776	Protein kinase
48	0	1,067	0	735	Deoxyribonuclease
49	24,761	346,248,208	1,384,850	2,616,008	Myristylated virion protein
50	10,457	709,187	18,789	453,678	Glycoprotein M
51	0	26,328	2,396	64,474	Origin binding protein
52	754	16,212	0	7,370	Component of DNA helicase–primase complex
53	14,317	2,041	211,526	35,602,303	Gamma-1 protein
54	2,018	9,984	0	2,171	Viral DNA cleavage/packaging
55	300,878	850,146	3,324	462,147	Component of DNA helicase–primase complex
56	1,507	147,681	2,540	65,285	Gamma-2 protein
57	5,991,973	74,351	2,876	16,357	Nonessential VZV protein
58	0	2,384	0	14,325	Phosphoprotein
59	0	4,932	0	3,958	Uracil DNA glycosylase
60	0	14,528	0	2,201	Glycoprotein L
61	2,460	15,162	2,176	45,144	Transcriptional activator IE1/latency-associated transcript
62	1,338	1,178	250	1,607	Transcriptional activator, IE3
63	198,530	6,824,892	224,147	47,283,036	Transcriptional activator, IE4
64	11,933	392,412	3,617	250,774	Tegument phosphoprotein
65	24,504	1,557,987	52,926	206,655	Tegument phosphoprotein
66	1,944	129,092	0	14,972	Protein kinase
67	9,220	453,634	5,600	149,243	Glycoprotein I
68	366	21,789	382	13,656	Glycoprotein E

Relative copy number per microgram total RNA

<sup>a</sup> Gray (2010)

and in 10 of 16 sensory ganglia in total. We also detected ORF B in 4 of 16 ganglia; ORFs A and 65 in 3 of 16 ganglia; ORF 68 in 2 of 16 ganglia; and ORFs 4, 10, 55, 63, 64, 66, and 67 in 1 of 16 latently infected sensory ganglia. Previous studies reported that the viral transactivator ORF 61 also expresses an antisense transcript in the ganglia of latently infected NHPs that is hypothesized to be the equivalent of the herpes simplex virus type-1 (HSV-1) LAT (Ou et al. 2007). Follow-up studies have shown that the frequency of the antisense transcripts greatly exceeds that of the sense transcripts in latently infected ganglia but not in infected Vero cells (Messaoudi et al. 2009; Ou et al. 2007). Our transcriptional analysis does not differentiate between the sense and antisense transcripts of ORF 61 but does support the previous data and suggests that the expression of ORF 61 plays an important role during the establishment or maintenance of SVV latency.

## Discussion

VZV and SVV are two closely related  $\alpha$ -herpesviruses that cause varicella in humans and nonhuman primates, respectively. VZV and SVV have the smallest genomes of the herpesvirus family. VZV encodes 68 ORFs, 3 novel ORFs, and 3 ORF duplications (Cohen et al. 2007; Kemble et al. 2000; McMillan et al. 1997; Ross et al. 1997). Similarly, SVV encodes 72 ORFs with 69 unique ORFs and 3 ORFs duplicated in the terminal repeat sequence. The SVV and VZV genomes differ at the left terminus where SVV encodes ORF A that is absent from VZV, and SVV lacks VZV ORF 2. ORF B of SVV is homologous to VZV ORF S/L. In this study, we examined SVV gene expression during acute and latent infection in rhesus macaques inoculated intrabronchially in order to identify genes that could play a critical role in the establishment and/or

**Table 4** SVV gene expression in PBMC at 10 dpi<sup>a</sup>

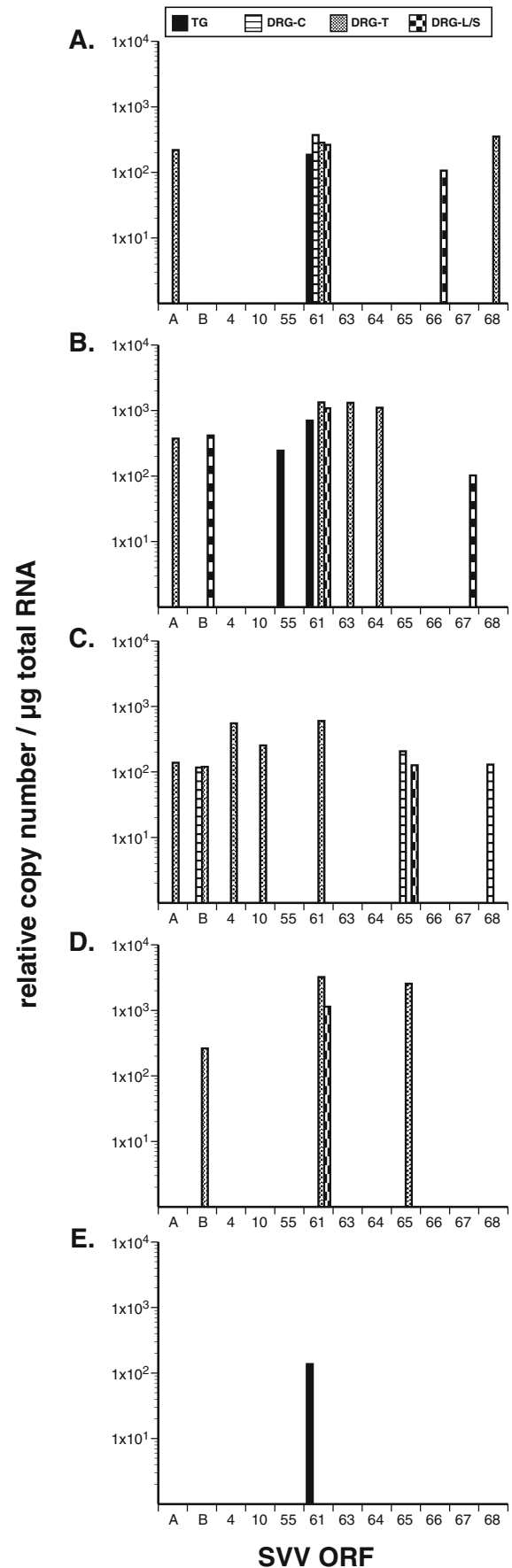
ORF	Animal ID		
	RM 20226	RM 24943	RM 25339
A	0	108	0
B	1,014	0	0
13	445	0	0
23	2,071	236	0
25	177	0	0
28	0	119	0
30	0	960	324
32	834	235	122
33	137	0	0
41	86,650	5,784	0
49	0	111	0
50	239	0	0
54	1,271	339	262
61	0	0	397
63	0	1,922	736
64	1,417	0	0
66	120	0	0
67	577	0	0

<sup>a</sup> Relative copy number per microgram total RNA

maintenance of latency. To the best of our knowledge, this is the first study to characterize the SVV transcriptome during acute and latent infection in tissues obtained from the same animal.

A comprehensive analysis of the *in vitro* acute SVV transcriptome using macroarrays showed that all SVV ORFs were expressed at 3 dpi in Vero cells, and ORF 9 was the most abundant SVV transcript detected followed by ORFs 32, 63, and 23 (Deitch et al. 2005). Likewise, our acute transcriptional analysis detected all SVV ORFs in BAL samples between 3 and 7 dpi. More specifically, in rhesus macaque 25043 (the animal with the highest overall gene expression), ORFs 49 and 41 were the most abundant transcripts in the BAL at 7 dpi, followed by ORFs 63 and 23. The top four ORFs detected in RM 25339 at 7 dpi include ORF 41, ORF 63, ORF 53, and ORF 7. Similarly, in BAL cells from RM 24953 at 7 dpi, which displayed a decreased transcriptional profile, SVV ORFs 41, 49, 63, and 53 were also the most abundantly expressed. However, in contrast to the *in vitro* macroarray study, ORF 32 was in the top ten highest expressing transcripts, but ORF 9 was

**Fig. 2** SVV transcriptome in latently infected sensory ganglia. Relative copy number from RT-qPCR analysis of SVV RNAs isolated from infected, trigeminal ganglia (TG), cervical dorsal root ganglia (DRG-C), thoracic dorsal root ganglia (DRG-T), and lumbar/sacral dorsal root ganglia (DRG-L/S) at >72 dpi in **a** RM 25043, **b** RM 25339, **c** RM 24953, **d** RM 20226, and **e** RM 24943





**Table 5** SVV ORFs expressed in latently infected sensory ganglia

ORF	# of samples positive/16	copy # <sup>a</sup>	Putative function or function based on known VZV or HSV-1 homology <sup>b</sup>
61	10	100–4,000	Transcriptional activator IE1/latency-associated transcript
B	4	<500	Homolog VZV ORF S/L: cytoplasmic viral protein, unknown function
A	3	<400	Truncated homolog ORF4, unknown function
65	3	100–3,000	Tegument phosphoprotein
68	2	<400	Glycoprotein E
4	1	<600	Transcriptional activator, IE2
10	1	<300	Transcriptional activator, tegument protein
55	1	<300	Component of DNA helicase–primase complex
63	1	<1,500	Transcriptional activator, IE4
64	1	<1,200	Tegument phosphoprotein
66	1	<200	Protein kinase
67	1	<200	Glycoprotein I

<sup>a</sup> Relative copy number range/microgram total RNA between 16 samples analyzed

<sup>b</sup> Gray WL (2010)

not in the top 20 though the relative expression exceeded  $2 \times 10^5$  copies. The difference in ORF 9 expression is most likely due to the cell type that SVV infected in vivo (lymphocytes and lung epithelial cells) versus the in vitro study, which utilized Veros (kidney epithelial cell line), which are interferon deficient, and a different cellular response to viral infection could modulate SVV gene expression.

Previous studies examined SVV gene expression in skin, lung, liver, spleen, and thoracic dorsal root ganglia samples harvested at 10–11 dpi in acutely infected St. Kitts vervet monkeys (*Cercopithecus aethiops*). Using RT-PCR, the study focused on ORFs 31, 36, 62, 63, and 68, which were detected in all tissues including the ganglia (Gray et al. 2002). Our analysis of PBMCs collected 10 dpi detected only 18 viral genes. ORFs 32 and 54 were detected in all three samples studied, and ORFs 23, 30, 41, and 63 were detected in two of three samples. ORF 23 (capsid), ORF 41 (capsid), and ORF 63 (transcriptional activator) were the most abundantly expressed genes in PBMC during acute infection. The differences between our observations and those of this earlier study could be due to differences in SVV pathogenesis. Vervet monkeys are highly susceptible to SVV infection, whereas rhesus macaques experience very low levels of SVV replication in peripheral blood (Soike et al. 1984). Moreover, we examined purified PBMC whereas this earlier study looked at spleen tissue, and it is likely that viral gene expression is cell-type specific (Streblov et al. 2007).

VZV acute transcriptional analysis has only been conducted in tissue culture to date. An in vitro macroarray study analyzed VZV lytic infection of BSC-1 cells and found that expression of transcripts associated with all VZV genes increased from 1 to 3 dpi, but the relative abundance did not change. The four

most abundant VZV ORFs were 9, 64, 33, and 49 (Cohrs et al. 2003b). VZV gene expression was also investigated by microarray in two additional cell lines, human melanoma (MeWo) and human astroglial (SVG) cells at 72 hpi (Kennedy et al. 2005). The results demonstrated that VZV transcriptional profiles differed between these two cell types and from BSC-1 cells. Sixty-eight of 71 VZV ORFs were detected in MeWo cells, and the most abundant ORFs were 57, 9, 49, 58, 48, and 69 while only 20 ORFs were detected in SVG cells and the most abundant ORFs were 24, 68, 61, 13, 32, and 53. The differences in copy numbers and ORFs detected could be due to the virus strains used and/or the cell types infected. For example, the VZV macroarray study used the Ellen strain of VZV in BSC-1, an African green monkey kidney cell line, whereas the microarray study utilized the Dumas strain of VZV in a melanoma cell line and an astroglial cell line. Interestingly, the SVV and VZV in vitro transcriptional studies using macroarrays identified ORF 9 as one of the most highly expressed genes (Cohrs et al. 2003b; Deitch et al. 2005). Both of the studies utilized cell lines derived from African green monkeys (Vero and BSC-1), which may influence the viral transcriptional program. In contrast, ORF 9 was not amongst the most abundant transcripts detected in vivo in the BAL or PBMC in our rhesus macaque model. This result emphasizes the value of not only studying the virus in single cell types but also in the context of the host.

Expression of SVV ORFs 21, 28, 29, 31, 61, 62, and 63 was characterized in vivo during latency by RT-PCR in liver, lung, trigeminal, cervical, and lumbar ganglia obtained from St. Kitts vervet monkeys (Ou et al. 2007). Only SVV ORF 21 and ORF 61 sense and ORF 61 antisense transcripts were detected in sensory ganglia. The expression of SVV ORFs was not detected in either the

lung or liver during latency. Another study using RNA ISH did not detect either SVV ORFs 21 or 63 in the ganglia of an African green monkey 2 months post-infection (Grinfeld and Kennedy 2007). As reported in our current study, we detected high levels of ORF 61 transcripts, but we did not detect ORF 21 above 100 copies, our established limit of detection. We also detected ORF 63 during latency, though only in one sample. The lack of expression of ORF 21 in our latently infected sensory could be due to differences in the monkey species (St. Kitts vervet or African green versus rhesus macaque) or the duration of latency. Animals in our study were euthanized at day 73 (RM 24953 and 25043), 77 (RM 24943 and 25339) or 178 (RM 20226) post-infection, the St. Kitts vervet monkeys in Ou et al. were euthanized at day 108 or 119 post-infection and in the Grinfeld et al. study, the African Green monkey was analyzed 2 months post-infection. Our rhesus macaques clear acute SVV infection between days 17 and 21 for a latency period of at least 2 months. While there are differences in the duration of latent infection between the studies, we propose that one of the major differences in SVV latent transcription is due to the nonhuman primate species utilized.

The most frequently detected gene in our analysis during latency was SVV ORF 61. SVV ORF 61 transactivates its own promoter as well as the promoters of immediate early gene ORF 62, early gene ORF 28 and 29, and late gene ORF 68 in transfected Vero cells (Gray et al. 2007). During latency, a LAT that is antisense to SVV ORF 61 was found to be expressed primarily in sensory ganglia (Ou et al. 2007). Both ORF 61 and LAT transcripts were detected in ganglia, but ORF 61 sense transcripts were detected most often during acute infection whereas LAT antisense transcripts were more abundant during latent infection (Ou et al. 2007). SVV ORF 61 is nonessential for replication *in vitro* (Gray et al. 2007), but its role *in vivo* remains to be determined. Together with the previous data and our current analysis, the data strongly suggest that ORF 61 plays an important role in the establishment and/or maintenance of SVV latency.

ORF B was the next most frequently detected transcript in 4 of 16 sensory ganglia collected from five SVV-infected rhesus macaques. ORF B is the homolog to VZV ORF S/L, which was recently determined to play a role in viral DNA cleavage (Kaufer et al. 2010). SVV ORF A and 65 were detected in 3 of 16 sensory ganglia. SVV ORF A is a truncation of SVV ORF 4 but has not been shown to transactivate viral genes like ORF 4 and has no homolog in VZV (Gray 2010). SVV ORF 65 is a tegument protein, and its VZV homolog was shown to be dispensable for viral replication in cell culture and *in vivo* (Cohen et al. 2001; Niizuma et al. 2003; Zhang et al. 2010). ORF 68 or glycoprotein E (gE) was detected in 2 of 16 ganglia, and the VZV homolog is essential for VZV replication *in vitro* (Mallory et al. 1998; Zhang et al. 2010). The gE protein is

involved in cell-to-cell spread and VZV infectivity (Ali et al. 2009; Zerbini et al. 2011) as well as immune evasion by interfering with complement activation and antibody-dependent cell-mediated cytotoxicity (Litwin et al. 1992).

SVV ORFs 4, 10, 55, 63, 64, 66, and 67 were only detected in 1 of 16 latent ganglia. SVV ORF 4 is a transcriptional activator, and the homolog in VZV is an essential gene for virus replication (Cohen et al. 2007). SVV ORF 10 is a viral transactivator and virion tegument protein. The VZV homolog was shown to be dispensable for growth in culture (MeWo), but a deletion virus showed a replication defect in skin organ cultures (Zhang et al. 2010). VZV and SVV ORF 55 have homology to HSV UL5, which is a component of the helicase–primase complex and is essential for viral DNA synthesis (Zhu and Weller 1988, 1992). SVV ORF 63/70 is a viral transcriptional activator, and its homolog in VZV is a tegument protein, and at least one gene copy is required for viral replication (Sommer et al. 2001). VZV ORF 63 is the most frequently detected transcript in latently infected human ganglia (Azarkh et al. 2010; Kennedy and Cohrs 2010) and is involved in the inhibition of the interferon- $\alpha$ -induced antiviral response (Ambagala and Cohen 2007). Furthermore, VZV ORF 63 was shown to inhibit apoptosis in primary human neurons thus promoting cell survival (Hood et al. 2006). VZV ORF 64/69 is a tegument phosphoprotein that is not essential for viral replication in culture but is associated with abnormal plaque phenotype (Sommer et al. 2001). VZV ORF 66 is a protein kinase (Eisfeld et al. 2006; Kinchington et al. 2000) that phosphorylates ORF 62, the main transactivator preventing nuclear accumulation, thus providing a potential mechanism to help maintain viral latency. VZV ORF 66 is also shown to be important for VZV infection of lymphocytes (Moffat et al. 1998; Schaap et al. 2005; Soong et al. 2000) and to play a role in MHC-I downregulation (Abendroth et al. 2001; Eisfeld et al. 2007). Finally, SVV ORF 67 or glycoprotein I (gI) is the binding partner for gE, and deletion of gI in VZV results in small plaque phenotype and is avirulent in a skin xenograft model (Mallory et al. 1997; Oliver et al. 2011). Studying the role of these SVV ORFs *in vivo* in the rhesus macaque model will yield critical insight into viral pathogenesis and latency.

The following observations highlight some differences between the transcriptional profiles observed during SVV and VZV latency. VZV ORF 63 is the most frequent and abundant transcript detected during latency in sensory ganglia (Cohrs and Gilden 2007). A recent study characterized the entire VZV transcriptome in latently infected human trigeminal ganglia using a multiplex RT-PCR approach (Nagel et al. 2011, 2009). In agreement with previous analyses, this study detected VZV ORFs 4, 29, 40, 62, and 63, and ORF 63 was the most prevalent transcript. Moreover, this approach also

detected ORFs 11, 41, 43, 57, and 68, which were not previously reported, but not ORFs 18, 21, and 66 previously detected in latently infected ganglia. ORF 66 has been detected in latently infected human ganglia by RT-dependent nested PCR, ISH, and real-time PCR (Cohrs and Gilden 2007; Cohrs et al. 2003a). Similar to the VZV latency analyses, our study detected SVV ORFs 4, 63, 66, and 68 in latent sensory ganglia albeit in very few ganglia. The VZV transcriptional studies did not detect ORF 61 transcripts during latency. This difference could be due to host factors influencing latency or different mechanisms employed by SVV and VZV to establish and/or maintain latency. VZV ORF 61 is expressed early during infection of human fibroblasts (Reichelt et al. 2009) and is important for VZV lytic replication in vitro. Specifically, in transient expression assays, ORF 61 enhanced VZV infectivity (Moriuchi et al. 1992). While deleting a large portion of ORF 61 impaired VZV replication in cell culture (Cohen and Nguyen 1998), total ORF 61 deletion resulted in complete loss of VZV replication (Zhang et al. 2010). Interestingly, both VZV ORF 21 and 61 were dispensable for the establishment of latency in the cotton rat model (Sato et al. 2003; Xia et al. 2003). These observations differ from what are observed in SVV where ORF 61 deletion has minimal effect on SVV replication in vitro (Gray et al. 2007) although the effect of VZV ORF 61 loss in the natural host remains unknown.

Our study furthers the understanding of lytic and latent gene transcription during SVV infection of rhesus macaques, a critical animal model of VZV infection. A detailed understanding of the molecular biology of SVV coupled with our robust animal model will aid the studies of VZV pathogenesis, antiviral therapy, and vaccines. Future studies will characterize the role of these genes in the establishment and maintenance of latency in vivo, a critical step in the understanding of viral and host factors that modulate reactivation.

**Acknowledgments** We would like to thank Anj Stadnik and Kyung Park for technical assistance; the Division of Animal Resources (DAR) at the Oregon National Primate Research Center for expert animal care, especially Drs. Anne Lewis and Lois Colgin for conducting the necropsies and collecting tissues; and Alfred Legasse, Miranda Fischer, and Jesse Dewayne for collection of blood and BAL samples. This work was supported by the American Heart Association career development grant 0930234N, NIH R01AG037042, 2T32AI007472-16, NIH P51 RR00163-51, and the Brookdale Foundation.

## References

- Abendroth A, Lin I, Slobedman B, Ploegh H, Arvin AM (2001) Varicella-zoster virus retains major histocompatibility complex class I proteins in the Golgi compartment of infected cells. *J Virol* 75:4878–4888
- Ali MA, Li Q, Fischer ER, Cohen JI (2009) The insulin degrading enzyme binding domain of varicella-zoster virus (VZV) glycoprotein E is important for cell-to-cell spread and VZV infectivity, while a glycoprotein I binding domain is essential for infection. *Virology* 386:270–279
- Ambagala AP, Cohen JI (2007) Varicella-zoster virus IE63, a major viral latency protein, is required to inhibit the alpha interferon-induced antiviral response. *J Virol* 81:7844–7851
- Azarkh Y, Gilden D, Cohrs RJ (2010) Molecular characterization of varicella zoster virus in latently infected human ganglia: physical state and abundance of VZV DNA, quantitation of viral transcripts and detection of VZV-specific proteins. *Curr Top Microbiol Immunol* 342:229–241
- Cohen JI, Nguyen H (1998) Varicella-zoster virus ORF61 deletion mutants replicate in cell culture, but a mutant with stop codons in ORF61 reverts to wild-type virus. *Virology* 246:306–316
- Cohen JI, Sato H, Srinivas S, Lekstrom K (2001) Varicella-zoster virus (VZV) ORF65 virion protein is dispensable for replication in cell culture and is phosphorylated by casein kinase II, but not by the VZV protein kinases. *Virology* 280:62–71
- Cohen JI, Straus SE, Arvin AM (2007) Varicella-zoster virus replication, pathogenesis, and management. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (eds) *Fields virology*. Lippincott Williams & Wilkins, Philadelphia, pp 2773–2818
- Cohrs RJ, Gilden DH (2007) Prevalence and abundance of latently transcribed varicella-zoster virus genes in human ganglia. *J Virol* 81:2950–2956
- Cohrs RJ, Barbour MB, Mahalingam R, Wellish M, Gilden DH (1995) Varicella-zoster virus (VZV) transcription during latency in human ganglia: prevalence of VZV gene 21 transcripts in latently infected human ganglia. *J Virol* 69:2674–2678
- Cohrs RJ, Barbour M, Gilden DH (1996) Varicella-zoster virus (VZV) transcription during latency in human ganglia: detection of transcripts mapping to genes 21, 29, 62, and 63 in a cDNA library enriched for VZV RNA. *J Virol* 70:2789–2796
- Cohrs RJ, Randall J, Smith J, Gilden DH, Dabrowski C, van Der Keyl H, Tal-Singer R (2000) Analysis of individual human trigeminal ganglia for latent herpes simplex virus type 1 and varicella-zoster virus nucleic acids using real-time PCR. *J Virol* 74:11464–11471
- Cohrs RJ, Gilden DH, Kinchington PR, Grinfeld E, Kennedy PG (2003a) Varicella-zoster virus gene 66 transcription and translation in latently infected human ganglia. *J Virol* 77:6660–6665
- Cohrs RJ, Hurley MP, Gilden DH (2003b) Array analysis of viral gene transcription during lytic infection of cells in tissue culture with varicella-zoster virus. *J Virol* 77:11718–11732
- Deitch SB, Gilden DH, Wellish M, Smith J, Cohrs RJ, Mahalingam R (2005) Array analysis of simian varicella virus gene transcription in productively infected cells in tissue culture. *J Virol* 79:5315–5325
- Eisfeld AJ, Turse SE, Jackson SA, Lerner EC, Kinchington PR (2006) Phosphorylation of the varicella-zoster virus (VZV) major transcriptional regulatory protein IE62 by the VZV open reading frame 66 protein kinase. *J Virol* 80:1710–1723
- Eisfeld AJ, Yee MB, Erazo A, Abendroth A, Kinchington PR (2007) Downregulation of class I major histocompatibility complex surface expression by varicella-zoster virus involves open reading frame 66 protein kinase-dependent and -independent mechanisms. *J Virol* 81:9034–9049
- Gray WL (2010) Simian varicella virus: molecular virology. *Curr Top Microbiol Immunol* 342:291–308
- Gray WL, Oakes JE (1984) Simian varicella virus DNA shares homology with human varicella-zoster virus DNA. *Virology* 136:241–246
- Gray WL, Starnes B, White MW, Mahalingam R (2001) The DNA sequence of the simian varicella virus genome. *Virology* 284:123–130
- Gray WL, Mullis L, Soike KF (2002) Viral gene expression during acute simian varicella virus infection. *J Gen Virol* 83:841–846

- Gray WL, Davis K, Ou Y, Ashburn C, Ward TM (2007) Simian varicella virus gene 61 encodes a viral transactivator but is non-essential for in vitro replication. *Arch Virol* 152:553–563
- Grinfeld E, Kennedy PG (2007) The pattern of viral persistence in monkeys intra-tracheally infected with Simian varicella virus. *Virus Genes* 35:289–292
- Hood C, Cunningham AL, Slobodman B, Arvin AM, Sommer MH, Kinchington PR, Abendroth A (2006) Varicella-zoster virus ORF63 inhibits apoptosis of primary human neurons. *J Virol* 80:1025–1031
- Kaufner BB, Smejkal B, Osterrieder N (2010) The varicella-zoster virus ORFS/L (ORF0) gene is required for efficient viral replication and contains an element involved in DNA cleavage. *J Virol* 84:11661–11669
- Kemble GW, Annunziato P, Lungu O, Winter RE, Cha TA, Silverstein SJ, Spaete RR (2000) Open reading frame S/L of varicella-zoster virus encodes a cytoplasmic protein expressed in infected cells. *J Virol* 74:11311–11321
- Kennedy PG, Cohrs RJ (2010) Varicella-zoster virus human ganglionic latency: a current summary. *J Neurovirol* 16:411–418
- Kennedy PG, Grinfeld E, Bell JE (2000) Varicella-zoster virus gene expression in latently infected and explanted human ganglia. *J Virol* 74:11893–11898
- Kennedy PG, Grinfeld E, Bontems S, Sadzot-Delvaux C (2001) Varicella-zoster virus gene expression in latently infected rat dorsal root ganglia. *Virology* 289:218–223
- Kennedy PG, Grinfeld E, Craigion M, Vierlinger K, Roy D, Forster T, Ghazal P (2005) Transcriptomal analysis of varicella-zoster virus infection using long oligonucleotide-based microarrays. *J Gen Virol* 86:2673–2684
- Kinchington PR, Fite K, Turse SE (2000) Nuclear accumulation of IE62, the varicella-zoster virus (VZV) major transcriptional regulatory protein, is inhibited by phosphorylation mediated by the VZV open reading frame 66 protein kinase. *J Virol* 74:2265–2277
- Litwin V, Jackson W, Grose C (1992) Receptor properties of two varicella-zoster virus glycoproteins, gpI and gpIV, homologous to herpes simplex virus gE and gI. *J Virol* 66:3643–3651
- Mahalingam R, Smith D, Wellish M, Wolf W, Dueland AN, Cohrs R, Soike K, Gilden D (1991) Simian varicella virus DNA in dorsal root ganglia. *Proc Natl Acad Sci USA* 88:2750–2752
- Mallory S, Sommer M, Arvin AM (1997) Mutational analysis of the role of glycoprotein I in varicella-zoster virus replication and its effects on glycoprotein E conformation and trafficking. *J Virol* 71:8279–8288
- Mallory S, Sommer M, Arvin AM (1998) Analysis of the glycoproteins I and E of varicella-zoster virus (VZV) using deletional mutations of VZV cosmids. *J Infect Dis* 178(Suppl 1):S22–S26
- McMillan DJ, Kay J, Mills JS (1997) Characterization of the proteinase specified by varicella-zoster virus gene 33. *J Gen Virol* 78(Pt 9):2153–2157
- Messaoudi I, Barron A, Wellish M, Engelmann F, Legasse A, Planer S, Gilden D, Nikolich-Zugich J, Mahalingam R (2009) Simian varicella virus infection of rhesus macaques recapitulates essential features of varicella zoster virus infection in humans. *PLoS Pathog* 5:e1000657
- Moffat JF, Zerboni L, Sommer MH, Heineman TC, Cohen JI, Kaneshima H, Arvin AM (1998) The ORF47 and ORF66 putative protein kinases of varicella-zoster virus determine tropism for human T cells and skin in the SCID-hu mouse. *Proc Natl Acad Sci USA* 95:11969–11974
- Moriuchi H, Moriuchi M, Smith HA, Straus SE, Cohen JI (1992) Varicella-zoster virus open reading frame 61 protein is functionally homologous to herpes simplex virus type 1 ICP0. *J Virol* 66:7303–7308
- Nagel MA, Gilden D, Shade T, Gao B, Cohrs RJ (2009) Rapid and sensitive detection of 68 unique varicella zoster virus gene transcripts in five multiplex reverse transcription-polymerase chain reactions. *J Virol Methods* 157:62–68
- Nagel MA, Choe A, Traktinskiy I, Cordery-Cotter R, Gilden D, Cohrs RJ (2011) Varicella-zoster virus transcriptome in latently infected human ganglia. *J Virol* 85:2276–2287
- Niizuma T, Zerboni L, Sommer MH, Ito H, Hinchliffe S, Arvin AM (2003) Construction of varicella-zoster virus recombinants from parent Oka cosmids and demonstration that ORF65 protein is dispensable for infection of human skin and T cells in the SCID-hu mouse model. *J Virol* 77:6062–6065
- Oliver SL, Sommer MH, Reichelt M, Rajamani J, Vlaycheva-Beisheim L, Stamatis S, Cheng J, Jones C, Zehnder J, Arvin AM (2011) Mutagenesis of varicella-zoster virus glycoprotein I (gI) identifies a cysteine residue critical for gE/gI heterodimer formation, gI structure, and virulence in skin cells. *J Virol* 85:4095–4110
- Ou Y, Davis KA, Traina-Dorge V, Gray WL (2007) Simian varicella virus expresses a latency-associated transcript that is antisense to open reading frame 61 (ICP0) mRNA in neural ganglia of latently infected monkeys. *J Virol* 81:8149–8156
- Oxman MN, Levin MJ, Johnson GR, Schmader KE, Straus SE, Gelb LD, Arbeit RD, Simberkoff MS, Gershon AA, Davis LE, Weinberg A, Boardman KD, Williams HM, Zhang JH, Peduzzi PN, Beisel CE, Morrison VA, Guatelli JC, Brooks PA, Kauffman CA, Pachucki CT, Neuzil KM, Betts RF, Wright PF, Griffin MR, Brunell P, Soto NE, Marques AR, Keay SK, Goodman RP, Cotton DJ, Gnann JW Jr, Loutit J, Holodniy M, Keitel WA, Crawford GE, Yeh SS, Lobo Z, Toney JF, Greenberg RN, Keller PM, Harbecke R, Hayward AR, Irwin MR, Kyriakides TC, Chan CY, Chan IS, Wang WW, Annunziato PW, Silber JL (2005) A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. *N Engl J Med* 352:2271–2284
- Pumphrey CY, Gray WL (1992) The genomes of simian varicella virus and varicella zoster virus are colinear. *Virus Res* 26:255–266
- Reichelt M, Brady J, Arvin AM (2009) The replication cycle of varicella-zoster virus: analysis of the kinetics of viral protein expression, genome synthesis, and virion assembly at the single-cell level. *J Virol* 83:3904–3918
- Ross J, Williams M, Cohen JI (1997) Disruption of the varicella-zoster virus dUTPase and the adjacent ORF9A gene results in impaired growth and reduced syncytia formation in vitro. *Virology* 234:186–195
- Sato H, Pesnicak L, Cohen JI (2003) Use of a rodent model to show that varicella-zoster virus ORF61 is dispensable for establishment of latency. *J Med Virol* 70(Suppl 1):S79–S81
- Schaap A, Fortin JF, Sommer M, Zerboni L, Stamatis S, Ku CC, Nolan GP, Arvin AM (2005) T-cell tropism and the role of ORF66 protein in pathogenesis of varicella-zoster virus infection. *J Virol* 79:12921–12933
- Soike KF, Rangan SR, Gerone PJ (1984) Viral disease models in primates. *Adv Vet Sci Comp Med* 28:151–199
- Sommer MH, Zagha E, Serrano OK, Ku CC, Zerboni L, Baiker A, Santos R, Spengler M, Lynch J, Grose C, Ruyechan W, Hay J, Arvin AM (2001) Mutational analysis of the repeated open reading frames, ORFs 63 and 70 and ORFs 64 and 69, of varicella-zoster virus. *J Virol* 75:8224–8239
- Soong W, Schultz JC, Patera AC, Sommer MH, Cohen JI (2000) Infection of human T lymphocytes with varicella-zoster virus: an analysis with viral mutants and clinical isolates. *J Virol* 74:1864–1870
- Streblov DN, van Cleef KW, Kreklywich CN, Meyer C, Smith P, Defilippis V, Grey F, Fruh K, Searles R, Bruggeman C, Vink C, Nelson JA, Orloff SL (2007) Rat cytomegalovirus gene expression in cardiac allograft recipients is tissue specific and does not parallel the profiles detected in vitro. *J Virol* 81:3816–3826

- Xia D, Srinivas S, Sato H, Pesnicak L, Straus SE, Cohen JI (2003) Varicella-zoster virus open reading frame 21, which is expressed during latency, is essential for virus replication but dispensable for establishment of latency. *J Virol* 77:1211–1218
- Zerboni L, Berarducci B, Rajamani J, Jones CD, Zehnder JL, Arvin A (2011) Varicella-zoster virus glycoprotein E is a critical determinant of virulence in the SCID mouse-human model of neuropathogenesis. *J Virol* 85:98–111
- Zhang Z, Selariu A, Warden C, Huang G, Huang Y, Zaccheus O, Cheng T, Xia N, Zhu H (2010) Genome-wide mutagenesis reveals that ORF7 is a novel VZV skin-tropic factor. *PLoS Pathog* 6:e1000971
- Zhu L, Weller SK (1988) UL5, a protein required for HSV DNA synthesis: genetic analysis, overexpression in *Escherichia coli*, and generation of polyclonal antibodies. *Virology* 166:366–378
- Zhu LA, Weller SK (1992) The six conserved helicase motifs of the UL5 gene product, a component of the herpes simplex virus type 1 helicase-primase, are essential for its function. *J Virol* 66:469–479