Available online at www.sciencedirect.com
SCIENCE DIRECT
Antiviral Research 68 (2005) 56–65
Antiviral Research
www.elsevier.com/locate/antiviral

Viral and cellular gene transcription in fibroblasts infected with small plaque mutants of varicella-zoster virus

Jeremy O. Jones *, Ann M. Arvin

Departments of Pediatrics and Microbiology and Immunology, Stanford University, 300 Pasteur Drive, Rm G312, Stanford, CA, USA

Received 20 April 2005; accepted 13 June 2005

Abstract

Varicella-zoster virus (VZV) is an alphaherpesvirus that causes varicella and herpes zoster. In these experiments, cDNA corresponding to 69 VZV open reading frames was added to 42K human cDNA microarrays and used to examine viral as well as cellular gene transcription concurrently in fibroblasts infected with two genetically distinct small plaque VZV mutants, rOka/ORF63rev[T171] and rOkaΔgI. rOka/ORF63rev[T171] has a point mutation in ORF63, which encodes the immediate early regulatory protein, IE63, and rOkaΔgI has a deletion of ORF67, encoding glycoprotein I (gI). rOka/ORF63rev[T171] was deficient in the transcription of several viral genes compared to the recombinant rOka control virus. Deletion of ORF67 had minimal effects on viral gene transcription. Effects of rOka/ORF63rev[T171] and rOkaΔgI on host cell gene transcription were similar to the rOka control, but a few host cell genes were regulated differently in rOkaΔgI-infected cells. Infection of fibroblasts with intact or small plaque VZV mutants was associated with down-regulation of NF-κB and interferon responsive genes, down-regulation of TGF-β responsive genes accompanied by reduced amounts of fibrotic/wound healing response genes (e.g. collagens, follistatin) and activation of cellular proliferation genes, and alteration of neuronal growth markers, as well as cellular genes encoding proteins important in protein and vesicle trafficking. These observations suggest that replication of VZV small plaque mutant viruses and intact VZV have similar consequences for host cell gene transcription in infected cells, and that the small plaque phenotype in these mutants reflects deficiencies in viral gene expression.

Keywords: Varicella-zoster virus; Fibroblast; Gene transcription

1. Introduction

Varicella-zoster virus (VZV) is a human alphaherpesvirus that causes varicella (chickenpox) and herpes zoster (Arvin, 2001; Cohen, 2001). VZV pathogenesis requires replication in skin, which appears to be mediated by transfer of infectious virus in T cells (Ku et al., 2004). cDNA microarrays have been used extensively to study host cell responses to viral pathogens. In previous work, we used microarrays to describe the effects of VZV recombinant parental and vaccine Oka viruses on cellular gene transcription in fibroblasts, primary tonsillar T cells, and human skin xenografts in SCIDhu mice (Jones and Arvin, 2003). Many studies have used microarrays to examine the host response to infection with herpesviruses, including human cytomegalovirus (HCMV) infection of primary human fibroblasts (Browne et al., 2001), HHV-6 infection of transformed T cells (Mayne et al., 2001), Epstein-Barr virus (EBV) infection of transformed B cells (Carter et al., 2002; Islam et al., 2002), Kaposi's sarcoma-associated herpesvirus (KSHV) infection of dermal microvascular endothelial cells (Moses et al., 2002), and HSV-1 infection of primary human fibroblasts (Mossman et al., 2001) and mouse trigeminal ganglia (Kramer et al., 2003; Tsavachidou et al., 2001). Viral gene transcription has also been investigated by microarray in BSC-1 cells infected with VZV (Cohrs et al., 2003), Hela cells infected with wild-type and UL54-deleted HSV-1 (Stingley et al., 2000), and in BCBL-1 cells infected with KSHV (Paulose-Murphy et al., 2001). These studies have demonstrated many commonalities in the host cell response to herpesviruses. In particular, interferon

* Corresponding author. Tel.: +1 650 725 6555; fax: +1 650 725 8040.
E-mail address: jjones@stanford.edu (J.O. Jones).

0166-3542/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2005.06.011

response genes were modulated by all herpesviruses that have been evaluated by microarray. Herpesvirus infections tend to affect the regulation of other immune system genes as well, including interleukins and other cytokines. Genes involved in basic cellular processes such as cell cycle control, transcription, and translation also tend to be altered by herpesvirus infection. Microarray data from several studies has demonstrated the activation of NF-κB responsive genes in herpesvirus-infected cells, and the persistent activation of the NF-κB pathway has been confirmed by other molecular techniques in HSV-1, HSV-2, CMV, EBV, and KSHV-infected cells (Atkinson et al., 2003; Cahir-McFarland et al., 2004; Caposio et al., 2004; Chaudhary et al., 1999; Field et al., 2003; Kowalik et al., 1993; Liu et al., 2002; Luftig et al., 2003; Matta and Chaudhary, 2004; Patel et al., 1998; Taddeo et al., 2003; Tsavachidou et al., 2001). Recent microarray studies involving herpesviruses have also shown that approximately one-third of cell genes were commonly regulated in fibroblasts infected with pseudorabiesvirus or HSV-1 (Ray and Enquist, 2004). Mutants with deletions of HSV-1 ICP27 (UL54) and viral host shutoff (UL41) proteins have also been studied to determine the differences in host cell responses to these genes (Stingley et al., 2000; Taddeo et al., 2002).

The microarrays presented in this study combined VZV cDNAs corresponding to 69 viral ORFs with a 42 K human cDNA array, in order to examine viral gene transcription and host cell responses to infection concurrently. We used the VZV/cellular gene microarrays to compare two VZV mutants, rOkaΔgI and rOka/63rev[T171], both of which have a small plaque phenotype in cell culture. While other VZV mutants display a similar phenotype, these two viruses were chosen for analysis because the mutations leading to the small plaque phenotype occur in proteins of distinct putative kinetic classes representing the breadth of viral gene transcription; IE63 is presumed to be expressed at immediate early times after infection, while gI is presumed to be expressed at late times after infection. The rOkaΔgI virus lacks the ORF67 gene, which encodes glycoprotein I (gI). Full and partial gI deletion mutants are infectious, although syncytium formation and virus yields are diminished (Mallory et al., 1997), and few virions reach post-Golgi structures, indicating that gI is important for assembly and cell-cell spread in cell culture (Wang et al., 2001). VZV gI forms heterodimers with gE, enhances gE endocytosis from plasma membranes and facilitates its trafficking to the trans-Golgi network (Alconada et al., 1998; Mo et al., 2002). Although rOkaΔgI replicates in cell culture, gI is necessary for infection of human skin and T cells in SCIDhu mice in vivo (Moffat et al., 2002).

IE63 is a small viral regulatory protein that is expressed at immediate-early times after infection. The rOka/63rev[T171] virus has a point mutation that changes a tyrosine residue to an alanine residue in the IE63 protein (Baiker et al., 2004). IE63, the homolog of HSV-1 ICP22, is a component of the viral tegument (Kinchington et al., 1995) and localizes to the nucleus during lytic infection (Debrus et al., 1995). IE63 is heavily phosphorylated and is a substrate for VZV ORF47 kinase and cellular kinases; its cellular localization depends on its phosphorylation state (Bontems et al., 2002; Kenyon et al., 2001; Stevenson et al., 1996). The ORF63 gene exists in two copies in the VZV genome, but a single copy at the native or a non-native site in the genome permits normal VZV replication in cell culture as well as in skin xenografts in SCIDhu mice (Sommer et al., 2001). The rOka/ORF63rev[T171] mutant has normal expression of IE62 and IE63, but ORF47 kinase expression is decreased. Replication of rOka/ORF63rev[T171] is reduced dramatically in skin, but not in T cell xenografts in SCIDhu mice (Baiker et al., 2004). In addition to comparing rOka/ORF63rev[T171] with the genetically distinct rOkaΔgI small plaque mutant in this report, the viral/cellular microarray analysis of rOka/ORF63rev[T171]-infected cells demonstrated that the point mutation in IE63 caused a decrease in the transcription of certain viral genes. This result suggested that intact IE63 acts as a viral transactivator, which had previously only been shown in vitro. These studies also added significantly to our knowledge of the host response to VZV infection of primary fibroblasts.

2. Materials and methods

2.1. Viruses and cells

Recombinant Oka and rOkaΔgI (Mallory et al., 1997), as well as rOka/ORF63rev[T171] (Baiker et al., 2004) and the rOka control virus rOka/ORF63rev were generated from vaccine Oka-based cosmids as described previously. These viruses were propagated in human foreskin fibroblasts (HFF) and used at less than ten passages. rOka/ORF63rev is a recombinant Oka virus with both copies of native ORF63 deleted and an insertion of a copy of ORF63 at the non-native AvrII site in the genome. This revertant is the appropriate control for rOka/ORF63rev[T171], which also has both copies of native ORF63 deleted and an insertion of the T171 mutant copy of ORF63 at the non-native AvrII site. Replication characteristics of the rOka/ORF63rev virus and rOka are indistinguishable in vitro and in vivo (Baiker et al., 2004; Sato et al., 2003). Infected cell suspensions were used to infect HFF monolayers for assays. Cells were grown in modified Eagle's medium (Cellgro) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 U penicillin/mL, and 50 μg streptomycin/mL at 37 °C.

2.2. Microarrays

Total RNA isolation, cDNA preparation, and microarray hybridization was carried out as described previously (Jones and Arvin, 2003). Two types of microarray experiments were performed. Type I array experiments involved the hybridization of cDNA from virally-infected cells against uninfected control cDNA, while Type II array experiments involved the hybridization of cDNA from cells infected with one strain

of virus against cDNA from cells infected with a different strain of virus. The 42K spot, human cDNA microarrays were purchased from the Stanford Functional Genomics Facility (www.microarray.org).

Spots corresponding to VZV ORFs were added in duplicate to the arrays at the Stanford Functional Genomics Facility. Primers were designed to amplify the largest, non-overlapping regions of known viral ORFs using recombinant vOka infected cell DNA as a template (Supplemental Table 1). These PCR products were spotted on the arrays along with the human cDNAs. These arrays were used examine differences in viral and cellular gene expression in HFFs infected with an equal inoculum (M.O.I. ∼ 0.01) of rOka control, rOka/ORF63rev[T171], or rOkaΔgI. RNA was harvested from monolayers in T75 flasks when 95–100% of the cells were infected, between 72 and 96 h post-infection. Prior to hybridization, arrays were post-processed according to the Stanford Functional Genomics Facility protocol. The arrays were scanned with a Gene Pix Scanner 4000 A (Axon Instruments Inc.) and analyzed with the Scanalyze program (Eisen, shareware, http://rana.lbl.gov/). All array experiments were performed in duplicate or triplicate.

2.3. Microarray data analysis

Data was entered into the Stanford Microarray Database (SMD) for normalization, filtering, and retrieval (Sherlock et al., 2001). The standard SMD normalization was selected for all arrays. The quality of the arrays and the data filtering criteria were determined as described previously (Jones and Arvin, 2003). At least two identical arrays were used for each comparison. The Significance Analysis of Microarrays (SAM) program (Tusher et al., 2001) was used to analyze the data as described previously (Jones and Arvin, 2003). One class response analyses were conducted to identify significant changes in gene regulation in response to a test virus as compared to the uninfected cell control, or to identify significant changes in gene regulation in two different virus samples hybridized against each other. Two class response analyses were used to identify significant changes in gene regulation in response to one test virus versus another. All of the raw data and SAM analyses can be viewed at http://cmgm.stanford.edu/∼jjones. The raw data can also be found on the SMD at http://genome-www5.stanford.edu/MicroArray/SMD.

2.4. Real-time RT-PCR

Primers and probes were designed using Assay by Design (Applied Biosystems) to specifically recognize sequences within ORFs 28, 47, 51, 62, 63, and 67. A pre-designed primer/probe set was used to detect human HPRT as a control gene. Total RNA was isolated from infected fibroblasts as described above, quantified, subjected to DNase treatment by a DNA-free kit (Ambion), and reverse-transcribed using the High Capacity cDNA Archive kit (Applied Biosystems). 2× PCR master mix and 20× primer/probe mix, as well as standards (in duplicate) and samples (in triplicate) were aliquoted into 96-well plates. Real-time PCR was completed using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Samples were referenced to plasmid standards with known copy numbers of viral genes and normalized to total RNA using the HPRT data.

2.5. Protein analysis

Total protein lysates were harvested from mock infected or rOka infected fibroblast monolayers (M.O.I. ∼ 0.1), or monolayers treated with 20 nM TNF-α (Genzyme) for 5 min in T25 flasks using PhosphoSafe lysis buffer (Novagen). Lysates from equal cell numbers were run on polyacrylamide gels, transferred to nitrocellulose membranes, blocked with 5% milk in TBS, and probed with primary antibodies against Thrombospondin-1 (NeoMarkers), MCP-1 (Fitzgerald Industries), or α-tubulin (Sigma) as a loading control. Blots were washed, probed with secondary antibodies conjugated to HRP, and exposed with an ECL plus Western Blotting Detection System (Amersham Biosciences). Blots were stripped between primary antibody exposures by treatment with 0.2 M NaOH for 15 min.

3. Results

3.1. Evaluation of the VZV/human cDNA microarrays

When cDNA from primary fibroblasts that were ∼95–100% infected with rOka control virus was applied to the microarrays, 66 of 70 viral spots, including the two ORF62 cDNA spots, were visualized. Transcription of ORFs 22, 25, 65, and 66, was not detected on the microarrays but could be detected by RT-PCR (data not shown), indicating that the microarrays were not sensitive enough to detect transcripts corresponding to these genes or that the spotting of these viral cDNAs was poor. As expected, cDNA from cells infected with rOkaΔgI did not hybridize to the ORF67 spots, indicating that the arrays had the capacity to detect the absence of a viral gene transcript. No viral transcripts were detected in samples harvested immediately after adding the infected cell inoculum to the uninfected monolayer, suggesting that viral transcripts in the inoculum did not influence microarray results for samples harvested at later time points.

3.2. Analysis of VZV gene transcription in fibroblasts infected with the small plaque mutant viruses, rOka/ORF63rev[T171] and rOkaΔgI

Viral gene transcription was analyzed in fibroblasts infected with the small plaque mutant and rOka control viruses. In addition to the four viral transcripts that could not be detected on any microarrays, transcripts of ORFs 9, 11, 28, 43, 44, 47, 51, and 67 were not detected by microarray analy-

Table 1
Real-time RT-PCR quantification of VZV gene transcription in cells infected with rOka control or rOKA/ORF63rev[T171]

Viral ORF (function and putative kinetic class)	ORF copy number/ng RNA and percent transcription relative to rOka control normalized to ORF63 transcript			
	rOka control		rOka/ORF63rev[T171]	
28 (polymerase, late)	1.9×10^5	100%	1.3×10^3	15%
47 (protein kinase, early)	8.1×10^6	100%	1.5×10^5	36%
51 (origin binding, late)	5.8×10^6	100%	4.3×10^4	15%
67 (glycoprotein I, late)	2.4×10^7	100%	1.8×10^5	15%

sis of rOka/ORF63rev[T171]-infected cells and ORF 28, 43, and 67 were not detected in rOkaΔgI-infected cells. Expression of each of these genes was detected by RT-PCR, except for ORF67 in experiments with rOkaΔgI (data not shown). Thus, the small plaque phenotype viruses produced amounts of these viral transcripts that were below the level of detection by microarray, whereas these viral gene transcripts could be detected by microarray in cells infected with rOka control virus, suggesting that they were more abundant in cells infected with intact rOka.

To quantify the effects on VZV transcription that were observed in microarray experiments, the transcription of ORFs 28, 47, 51, 62, 63, and 67 was evaluated at late stages of infection in cells inoculated with the T171 mutant and the intact rOka control virus by real-time RT-PCR. TaqMan primers and probes were used to quantify the absolute amounts of viral transcripts, using known copy numbers of viral genes as a control and normalizing to cellular HPRT levels. To assess the specific transcriptional deficiencies of the rOka/ORF63rev[T171] mutant, viral gene transcription was normalized to the amount of ORF63 transcript present in each sample, since this transcript was detected similarly on each viral microarray, and IE63 protein expression was previously shown to be unaffected by the T171 mutation in IE63 (Baiker et al., 2004). By RT-PCR, transcription of ORFs 28, 47, 51, and 67 in rOka/ORF63rev[T171]-infected cells was decreased 64% or more, relative to transcription in rOka control infected cells when normalized to the amount of ORF63 transcript in each sample (Table 1). Similar results were obtained when transcription was normalized to ORF62 transcript (data not shown). These experiments suggested that the point mutation at amino acid 171 in IE63 protein affected its viral regulatory activity. As expected, the transcription of ORF67 was absent (<1%) in rOkaΔgI-infected cells. The transcription of ORFs 28 and 51 was also reduced compared to rOka control, but not as severely as in rOka/ORF63rev[T171]-infected cells (data not shown). While the transcription of ORF43 was not analyzed by RT-PCR, the transcription of ORF62 was also found to be reduced in rOkaΔgI-infected cells compared to the rOka control infected cells. The difference in ORF62 transcription likely reflected a small difference in the absolute number of infected cells between the rOkaΔgI and rOka control infected fibroblast monolayers used for analysis. This difference in the number of infected cells, or a reduced amount of IE62, the primary viral transactivator, likely account for the reduction in the amount of other viral transcripts; therefore, the differences in ORF28 and ORF51 trancription are not likely to be biologically significant.

3.3. Analysis of host cell gene transcription in fibroblasts infected with rOka/ORF63rev[T171]

In general, patterns of host transcriptional response in fibroblasts infected with the small plaque mutants, rOka/ORF63rev[T171], or rOkaΔgI, were found to be similar to the effects of rOka control virus. In order to accurately identify the host cell transcription pattern in response to infection, microarray comparisons were carried out in two ways. cDNA from an equivalent number of infected cells was hybridized against cDNA from mock-infected cells (Type 1 microarray comparisons) and the results were filtered and examined for significance using the SAM software. The fold change of significantly regulated genes found using this type of comparison are shown in Table 2. Experiments in which cDNA from rOka/ORF63rev[T171] infected cells was hybridized against rOka control infected cells (Type 2 microarray comparisons) were used to confirm and add to the findings derived from Type 1 comparisons. The significantly regulated genes confirmed by Type 2 comparisons are shown in the right-most column of Table 2, as denoted by a "+." The absence of confirmation of a gene by the Type 2 analysis simply implies that data for that particular gene did not pass our strict filtering requirements; no genes were found to be oppositely regulated by the two different microarray analysis strategies. While Table 2 does not included an exhaustive list of genes found to be significantly regulated by Type 1 or Type 2 microarray comparisons of rOka control and rOka/ORF63rev[T171]-infected cells, it does include groups of genes that are responsive to specific cellular signaling pathways or genes that could be involved in other specific virus–host cell interactions. Many genes known to be activated in response to NF-κB signaling were down-regulated in fibroblasts infected with rOka control or rOka/ORF63rev[T171] (Table 2A). Additional NF-κB responsive genes were found to be down-regulated by the Type 2 microarray analysis, including IL-1R1, IL-6, CCL7, GSTA4, PTEN, CXCL2, and CXCL3. NF-κB mediates multiple cellular processes, but is particularly important in the ability of the cell to mount an inflammatory response and initiate immune system signaling. The observation that NF-κB responsive genes were down-regulated in VZV-infected cells suggested that VZV can inhibit the NF-κB

Table 2
Analysis of host cell gene transcription in fibroblasts using rOka control or rOKA/ORF63rev[T171] infected cell cDNA hybridized against mock-infected cell cDNA

Gene	rOka control	rOKA/ORF63rev [T171]	Confirmed by Type 2 analysis
A: NF-κB responsive inflammatory genes			
SOD2	−1.68	−1.72	+
CYP1B1	−3.25	−1.02	+
Thrombospondin1	−5.17	−12.38	+
CCL2 (MCP-1)	−3.34	−10.56	+
STAT1	−2.20	−2.55	+
IL-1 beta	−1.48	−1.39	+
Collectin12	−1.87	−1.39	+
CXCL1	−2.51	−1.34	+
CXCL6	−1.39	−1.21	ND
CXCL12	−1.60	−5.58	+
TNFRSF11B	−2.51	−3.51	+
BIGM103	−1.25	ND	+
CSF1	ND	−2.51	+
TNFAIP2	ND	−1.56	+
TNFAIP3	ND	−1.53	+
IL-1R2	ND	−1.11	+
B: Interferon-stimulated genes			
IFIT1	−5.26	−7.03	+
IFNGR1	−1.01	−1.08	+
IFNGR2	1.25	−1.40	ND
IFITM1	−1.63	−1.14	+
IFITM2	ND	−1.15	+
IFIT2	−1.88	−1.02	ND
C: Other genes involved in immune response			
CD26	−1.20	−1.49	+
MASP1	−1.35	−2.23	+
Collectin12	−1.87	−1.39	+
Caspase 1	−2.42	ND	+
IRF2	ND	−9.33	+
SOCS5	−3.34	−2.98	+
D: TGF-β responsive and effector genes			
TGF-β responsive			
Col1A1	−3.63	−15.15	+
Col1A2	−2.14	−4.74	+
Col3A1	−3.31	−18.72	+
Col5A2	−1.96	−4.04	ND
ADAM12	−1.63	−2.83	+
Lumican	−1.78	−2.40	+
p27	−1.44	−1.59	ND
Self-limiting genes			
Id2	1.45	2.30	ND
Trans effector genes			
THBS1	−5.17	−12.38	+
THBS2	−2.34	−2.87	ND
CKTSF1B1	−3.03	−7.89	+
Follistatin	−1.08	−1.22	+
Follistatin-like1	−1.75	−2.46	ND
TGFBR2	−1.04	−1.29	ND
E: Genes involved with neuronal growth and development			
SEMA5A	−1.49	−1.71	+
PBX1	−2.97	−3.37	+
GAS1	ND	−6.90	+
PRG1	ND	−4.18	+
Myb	−2.31	−1.41	+
Meis2	−1.95	−2.77	+
F: Genes involved in vesicle and protein trafficking			
SYTL2	−2.52	−2.03	ND
VAMP4	−1.50	−3.25	+
SNX13	−2.22	ND	+
ABCC1	−1.78	−1.35	ND

The numbers in the second and third columns are the log (base 2) fold change of genes found to be significantly regulated by SAM analysis which come from the Type 1 microarray analysis. Genes confirmed by Type 2 microarray analysis are indicated with a +. ND: no data; data did not pass filtering requirements, or was not found to be significant by SAM analysis.

signaling pathway. Genes known to be stimulated by interferon were also down-regulated in infected cells (Table 2B), and another interferon response gene, Mx-1, was found to be similarly down-regulated by the Type 2 microarray analysis. As NF-κB can activate the transcription of interferon genes, the decrease in interferon responsive gene transcription could be due to an inhibition of NF-κB signaling (Jobin and Sartor, 2000; Parikh et al., 1997). Several other genes important for immune control of virus infection were down-regulated in VZV infected fibroblasts as well (Table 2C).

The TGF-β pathway, which controls multiple cell processes including the fibrotic, or wound healing, response (Verrecchia and Mauviel, 2002), was also affected by VZV infection (Table 2D). Known fibrosis genes, including various collagens, were down-regulated in infected cells. TGF-β can also arrest cell cycle progression in G1 by induction of the cyclin-dependent kinase inhibitor $p27^{kip}$ (Pierelli et al., 2000), which was down-regulated in VZV infected cells. Id2, a protein that prevents cell cycle exit (Ruzinova and Benezra, 2003) and whose transcription is normally decreased in response to TGF-β, was up-regulated by VZV infection. Likewise, Id1 was found to be up-regulated by the Type 2 microarray analysis. From this data, it appears that VZV was able to inhibit the transcription of TGF-β responsive genes, possibly by interfering with TGF-β signaling.

TGF-β signaling itself is initiated by interaction of TGF-β superfamily members TGF-β, activin, and bone marrow progenitor (BMP) with the TGF-β receptors 1 and 2 (Massague, 1998). TGF-β must be processed from its latent form (LTGF-β) to become active (Roberts, 1998). Thrombospondin proteins are major activators of LTGF-β, and both thrombospondins 1 and 2 were found to be highly down-regulated in VZV infected cells. Without thrombospondin-initiated activation of LTGF-β, signaling through this pathway should be inhibited, which was reflected in the microarray data. However, follistatin and the cysteine knot containing protein CKTSF1B1, which are inhibitors of the TGF-β family proteins activin and BMP, respectively, were also both down-regulated in VZV infected cells. This could possibly lead to the activation of the TGF-β pathway, but does not appear to do so in VZV-infected cells. Thus, the regulation of the TGF-β pathway by VZV infection is likely to be complex.

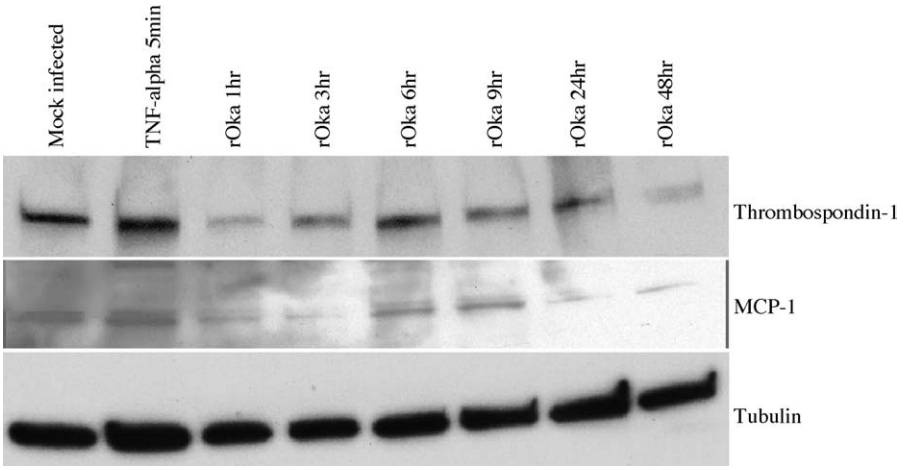

Fig. 1. Infection of fibroblasts with rOka control reduces expression of MCP-1 and Thrombospondin-1. Total cell lysate from equal numbers of mock-infected, TNF-α treated, or fibroblasts infected with rOka for the times indicated was probed with antibodies against the indicated proteins. The expression of Tubulin protein was used as a loading control.

Several genes involved in cell growth and development, especially neuronal cell growth, were down-regulated by VZV infection (Table 2E). Viral regulation of these genes may be important for viral replication and possibly establishment of latency in neurons, as well as growth in primary fibroblasts. Finally, transcription of genes involved protein and vesicle trafficking was decreased in VZV-infected cells (Table 2F). These genes may be important in virion formation and transport to sites of egress.

To ascertain whether the microarray findings could predict the protein levels of some of the genes that were found to be significantly regulated, total cell lysates were collected from rOka control infected monolayers and the presence of MCP-1 (CCL2) and Thrombospondin-1 were analyzed by western blotting. The levels of each of these proteins initially increase in the monolayer in response to viral infection, but severely decline by late time points, when most of the cells in the monolayer are infected, and when RNA was harvested for microarray analysis (Fig. 1). The level of these proteins also increased in response to TNF-α treatment, a potent inducer of the NF-κB pathway and NF-κB responsive genes such as MCP-1 and Thrombospondin-1. The changes in protein products of these genes correspond with the microarray results, though they occur at earlier time points because of differences in experimental design.

3.4. Unique effects of rOkaΔgI on host cell gene transcription

Although regulation of host cell transcription was similar among rOka control, rOka/ORF63rev[T171], and rOkaΔgI viruses, two-class SAM analyses revealed a few significant differences between fibroblasts infected with rOka/ORF63rev[T171] and rOkaΔgI for 72 h (Table 3). Significance was based on a software predicted ratio of 49 true positives to 2 false positives. The SAM score and the average log (base 2) fold regulation of each annotated gene are shown for the two small plaque mutants. Repeated genes indicate that multiple independent spots on the microarray for that gene were found to be affected significantly in this analysis. The transcription of a number of genes involved in cell signaling pathways, including a protein tyrosine phosphatase, a kinase, and the transcription factors maf and myc, are increased in rOkaΔgI-infected cells relative to rOka/ORF63rev[T171]-infected cells, suggesting that the lack of gI may alter certain signaling pathways in response to VZV infection. A SAM two-class analysis showed that the regulation of these host cell genes in rOkaΔgI infected cells also differed from that in rOka control infected cells (data not shown).

4. Discussion

The addition of VZV cDNAs to the human cDNA microarrays allowed for the concurrent assessment of viral transcription and the host cell response to VZV infection in fibroblasts infected with VZV recombinants expressing intact viral proteins, or with either of two small plaque mutants, rOkaΔgI and rOka/ORF63rev[T171]. Mutation of IE63 at amino acid 171 was associated with decreased levels of transcription of ORFs 9, 11, 28, 43, 44, 47, 51, and 67, as shown by microarray and confirmed by RT-PCR. Some of these genes encode proteins that are known to be important for viral growth and replication, such as ORF28, the large subunit of the viral DNA polymerase, ORF51, the origin binding protein, and ORF67, glycoprotein I. Transcription of two of these genes, ORFs 28 and 43, was also decreased in the absence of gI expression; as expected ORF67 was not detected. With few exceptions, the effects of rOka/ORF63rev[T171] and rOkaΔgI infection on host cell gene transcription were similar to the control rOka control virus. These observations suggest that replication of VZV small plaque mutant viruses and intact VZV have similar consequences for host cell gene transcription in infected cells and that the small plaque phenotype is related

Table 3
Host cell genes specifically regulated in response to rOkaΔgI virus infection

Gene	SAM score	rOkaΔgI average $\log_2$ regulation	rOka/ORF63rev[T171] average $\log_2$ regulation
CCL2	1.558	0.8235	3.7125
SLC15A2||solute carrier family	1.410	−0.063	2.7165
DKK1||dickkopf homolog	1.337	−1.041	1.23
CTGF||connective tissue growth factor	1.250	−3.2875	−0.6515
CTGF||connective tissue growth factor	1.222	−3.555	−0.888
CCL2	1.152	−0.185	2.3295
L1CAM	1.125	−0.9475	1.113
CDK7	1.113	−1.033	1.082
IGFBP7	1.050	−1.0765	1.317
LOX||lysyl oxidase	0.958	−0.212	1.365
SFRS10||splicing factor	1.193	1.402	−1.1135
TYRP||tyrosinase-related	1.135	2.6375	0.415
DNAJB1||DnaJ homolog	1.048	2.1965	−0.136
PGK1||phosphoglycerate kinase 1	1.022	1.338	−0.579
PYG||glycogen phosphorylase	1.021	1.179	−0.562
CALM3||calmodulin 3	1.020	0.2665	−1.7535
YWHAQ|	1.010	1.5355	−0.5875
NASP||nuclear autoantigenic sperm prt	0.988	0.662	−1.212
MAFG||v-maf homolog	0.979	0.598	−1.225
DEGS|| degenerative spermatocyte hom	0.978	1.096	−0.623
UBAP2||ubiquitin associated protein 2	0.958	0.837	−0.751
TEBP||unactive progesterone receptor	0.936	0.8455	−1.059
PRDX4||peroxiredoxin 4	0.928	0.1855	−1.8505
PTPN12||protein tyrosine phosphatase	0.918	0.9115	−0.646
H2AFX||H2A histone family	0.917	0.388	−1.7355
P4HA1||procollagen-proline	0.916	0.7925	−1.17
SF3B2||splicing factor 3b	0.908	0.5765	−1.152
PPIF||peptidylprolyl isomerase F	0.906	1.367	−0.309
NCOA4||nuclear receptor coactivator 4	0.901	0.3045	−1.364
EP300||E1A binding protein p300	0.897	0.721	−0.8665
PPIF||peptidylprolyl isomerase F	0.883	1.6535	−0.6605
MYC	0.853	1.111	−0.8015
MOXD1||monooxygenase	0.849	1.3145	−0.309
OXCT||3-oxoacid CoA transferase	0.838	0.9415	−1.37

to a deficit in viral gene transcription, rather than differences in host cell response. The rOkaΔgI virus expresses no gI and transcription of ORF67, which encodes gI, is much reduced in rOka/ORF63rev[T171]-infected cells. VZV gI is a critical determinant of the trafficking of gE to plasma membranes and the formation of gE:gI heterodimers is involved in cell fusion. Thus, the small plaque phenotypes could possibly be explained by the absence of gI in rOkaΔgI infected cells and its restricted synthesis in rOka/ORF63rev[T171]-infected cells.

Differing assessments of the contributions of the ORF63 gene product to VZV replication have been reported, based on transient expression experiments. The analysis of the IE63 mutant virus, rOka/ORF63rev[T171] with the VZV gene microarray allowed us to investigate this question in the context of VZV replication. Studies using co-transfection of viral protein expression vectors have indicated that IE63 can repress transcription of ORF62 while enhancing transcription of ORF36 (Jackers et al., 1992). However, other transient assays have found no clear role for IE63 as a viral transactivator/repressor (Kost et al., 1995). More recent studies have shown that transient IE63 expression can repress ORF28 and ORF36 promoter activity and that mutation of ten casein kinase I and II phosphorylation sites, including the T → A substitution at amino acid 171, can abrogate this repression activity in a cell type dependent manner (Bontems et al., 2002). Our microarray experiments in primary fibroblasts indicated that modification of IE63 by substituting alanine for the threonine at amino acid 171 resulted in the decreased transcription of some viral genes. The T171 mutation of IE63 was associated with decreased ORF47 kinase expression by immunoblot (Baiker et al., 2004), and the transcriptional analysis of ORF47 by microarray and RT-PCR was consistent with this observation. Overall, the evidence from these experiments is that IE63 may be involved in the transactivation of at least eight VZV genes, including ORFs 9, 11, 28, 43, 44, 47, 51, and 67, and that the mutation at amino acid 171 disrupts its transactivating activity. The differences identified for the role of IE63 in viral gene regulation between this study and other studies, likely reflects the fact that other studies have used transient expression systems while this study analyzed IE63 function in the context of viral replication.

By studying the rOka control and small plaque viruses, we added significantly to the data from our previous microarray analyses of the effects of VZV infection on cell gene expression in primary human fibroblasts (Jones and

Arvin, 2003). Though the microarray results presented in this report do not include an exhaustive list of fibroblasts genes affected by VZV infection as in the previous study, the two sets of microarray data were very similar (data not shown). The results from the current investigation made it possible to place more genes into the appropriate functional groups, and to better characterize specific pathways affected by VZV infection. VZV down-regulated NF-κB responsive gene transcription in infected cells, which suggests that the NF-κB pathway is inhibited in VZV infected cells. Infection with other herpesviruses, including HSV-1, HSV-2, CMV, EBV, and KSHV, causes the persistent activation and nuclear translocation of NF-κB proteins (Cahir-McFarland et al., 2004; Taddeo et al., 2003; Tsavachidou et al., 2001). NF-κB proteins translocate to the nucleus where they bind to specific κB elements and activate transcription of variety of genes encoding adhesion molecules, inflammatory and chemotactic cytokines, cytokine receptors, and enzymes that produce inflammatory mediators (Pahl, 1999). NF-κB is also a major transactivator of the interferon α/β genes. It is likely that the down-regulation of interferon responsive genes observed by microarray in VZV infected cells is directly related to the disruption of NF-κB signaling. HSV requires constitutive NF-κB activation for full viral replication and has evolved mechanisms for interfering with antiviral pathways initiated by NF-κB, including the virus host shut-off protein (vhs), which can degrade transcripts induced by NF-κB (Patel et al., 1998; Taddeo et al., 2002). Although VZV ORF17 encodes a protein with some amino acid homology to the vhs protein, VZV replication does not appear to have comparable effects on host cell protein synthesis (Sato et al., 2002). Therefore, the ability to disrupt NF-κB signaling and the immune response it evokes would be very beneficial to the growth and survival of the virus. We are currently investigating the mechanism by which VZV disrupts NF-κB signaling.

The interruption of TGF-β signaling in VZV-infected cells may be beneficial to the growth of the virus as well. For optimal replication and lytic growth, VZV requires cells to be in an active stage of the cell cycle. Since TGF-β inhibits cell cycle progression, blocking this inhibition should enhance viral replication. Limiting the wound-healing response could enhance the spread of the virus across the monolayer by inhibiting the repair of damaged cells.

The variety of the host cell genes that were regulated differently in response to rOkaΔgI infection and rOka/63rev[T171] infection make it difficult to determine specific pathways that may be differentially affected by infection with this mutant virus. As such, any further interpretation would be speculative. However, the dataset comparing the host cell transcriptional response to rOka and rOkaΔgI provides interesting leads that may be related to several biological differences between the viruses. Since gI is normally located in the plasma membrane of infected cells, interactions between gI and cell signaling proteins on the surface of cells may elicit specific transcriptional regulation of host cell genes that does not occur in the absence of gI in rOkaΔgI-infected cells. Alternatively, the absence of gI causes an accumulation of gE in the golgi and ER of infected cells, which could trigger an ER stress response and alter the transcription pattern of infected host cells.

We have determined a role for ORF63 in the transactivation of viral genes and shown that the cause of the small plaque phenotype is likely due to a deficiency in viral gene synthesis, not the host cell response, as host cell transcriptional responses in small plaque mutant virus infected cells were very similar to control Oka infected cells. However, we have identified a few specific host cell genes that are differentially regulated during infection with a virus lacking the gI protein.

Acknowledgments

This work was supported by grants from the National Institute of Allergy and Infectious Diseases, AI20459 and AI053846 (A.M.A.) and a grant from the National Science Foundation (J.O.J.). The authors would like to thank Carol Jones for assistance with real-time RT-PCR and the laboratory of Stanley Falkow for use of a microarray scanner. We also thank Chia-Chi Ku, Leigh Zerboni, and Marvin Sommer for technical assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.antiviral.2005.06.011.

References

Alconada, A., Bauer, U., Baudoux, L., Piette, J., Hoflack, B., 1998. Intracellular transport of the glycoproteins gE and gI of the varicella-zoster virus. gE accelerates the maturation of gI and determines its accumulation in the trans-Golgi network. J. Biol. Chem. 273, 13430–13436.

Arvin, A., 2001. Varicella-zoster virus. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology, fourth ed. Lippincott-Raven, Philadelphia, PA, pp. 2731–2768.

Atkinson, P.G., Coope, H.J., Rowe, M., Ley, S.C., 2003. Latent membrane protein 1 of Epstein-Barr virus stimulates processing of NF-kappa B2 p100 to p52. J. Biol. Chem. 278, 51134–51142.

Baiker, A., Bagowski, C., Ito, H., Sommer, M., Zerboni, L., Fabel, K., Hay, J., Ruyechan, W., Arvin, A.M., 2004. The immediate-early 63 protein of Varicella-Zoster virus: analysis of functional domains required for replication in vitro and for T-cell and skin tropism in the SCIDhu model in vivo. J. Virol. 78, 1181–1194.

Bontems, S., Di Valentin, E., Baudoux, L., Rentier, B., Sadzot-Delvaux, C., Piette, J., 2002. Phosphorylation of varicella-zoster virus IE63 protein by casein kinases influences its cellular localization and gene regulation activity. J. Biol. Chem. 277, 21050–21060.

Browne, E.P., Wing, B., Coleman, D., Shenk, T., 2001. Altered cellular mRNA levels in human cytomegalovirus-infected fibroblasts: viral block to the accumulation of antiviral mRNAs. J. Virol. 75, 12319–12330.

Cahir-McFarland, E.D., Carter, K., Rosenwald, A., Giltnane, J.M., Henrickson, S.E., Staudt, L.M., Kieff, E., 2004. Role of NF-kappa B in cell survival and transcription of latent membrane protein 1-expressing or Epstein-Barr virus latency III-infected cells. J. Virol. 78, 4108–4119.

Caposio, P., Dreano, M., Garotta, G., Gribaudo, G., Landolfo, S., 2004. Human cytomegalovirus stimulates cellular IKK2 activity and requires the enzyme for productive replication. J. Virol. 78, 3190–3195.

Carter, K.L., Cahir-McFarland, E., Kieff, E., 2002. Epstein-barr virus-induced changes in B-lymphocyte gene expression. J. Virol. 76, 10427–10436.

Chaudhary, P.M., Jasmin, A., Eby, M.T., Hood, L., 1999. Modulation of the NF-kappa B pathway by virally encoded death effector domains-containing proteins. Oncogene 18, 5738–5746.

Cohen, J.I., Straus, S.E., 2001. Varicella-zoster virus and its replication. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology, fourth ed. Lippincott-Raven, Philadelphia, PA, pp. 2703–2707.

Cohrs, R.J., Hurley, M.P., Gilden, D.H., 2003. Array analysis of viral gene transcription during lytic infection of cells in tissue culture with Varicella-Zoster virus. J. Virol. 77, 11718–11732.

Debrus, S., Sadzot-Delvaux, C., Nikkels, A.F., Piette, J., Rentier, B., 1995. Varicella-zoster virus gene 63 encodes an immediate-early protein that is abundantly expressed during latency. J. Virol. 69, 3240–3245.

Field, N., Low, W., Daniels, M., Howell, S., Daviet, L., Boshoff, C., Collins, M., 2003. KSHV vFLIP binds to IKK-gamma to activate IKK. J. Cell Sci. 116, 3721–3728.

Islam, T.C., Lindvall, J., Wennborg, A., Branden, L.J., Rabbani, H., Smith, C.I., 2002. Expression profiling in transformed human B cells: influence of Btk mutations and comparison to B cell lymphomas using filter and oligonucleotide arrays. Eur. J. Immunol. 32, 982–993.

Jackers, P., Defechereux, P., Baudoux, L., Lambert, C., Massaer, M., Merville-Louis, M.P., Rentier, B., Piette, J., 1992. Characterization of regulatory functions of the varicella-zoster virus gene 63-encoded protein. J. Virol. 66, 3899–3903.

Jobin, C., Sartor, R.B., 2000. The I kappa B/NF-kappa B system: a key determinant of mucosalinflammation and protection. Am. J. Physiol. Cell Physiol. 278, 451–462.

Jones, J.O., Arvin, A.M., 2003. Microarray analysis of host cell gene transcription in response to varicella-zoster virus infection of human T cells and fibroblasts in vitro and SCIDhu skin xenografts in vivo. J. Virol. 77, 1268–1280.

Kenyon, T.K., Lynch, J., Hay, J., Ruyechan, W., Grose, C., 2001. Varicella-zoster virus ORF47 protein serine kinase: characterization of a cloned, biologically active phosphotransferase and two viral substrates, ORF62 and ORF63. J. Virol. 75, 8854–8858.

Kinchington, P.R., Bookey, D., Turse, S.E., 1995. The transcriptional regulatory proteins encoded by varicella-zoster virus open reading frames (ORFs) 4 and 63 but not ORF 61 are associated with purified virus particles. J. Virol. 69, 4274–4282.

Kost, R.G., Kupinsky, H., Straus, S.E., 1995. Varicella-zoster virus gene 63: transcript mapping and regulatory activity. Virology 209, 218–224.

Kowalik, T.F., Wing, B., Haskill, J.S., Azizkhan, J.C., Baldwin Jr., A.S., Huang, E.S., 1993. Multiple mechanisms are implicated in the regulation of NF-kappa B activity during human cytomegalovirus infection. Proc. Natl. Acad. Sci. U.S.A. 90, 1107–1111.

Kramer, M.F., Cook, W.J., Roth, F.P., Zhu, J., Holman, H., Knipe, D.M., Coen, D.M., 2003. Latent herpes simplex virus infection of sensory neurons alters neuronal gene expression. J. Virol. 77, 9533–9541.

Ku, C.C., Zerboni, L., Ito, H., Graham, B.S., Wallace, M., Arvin, A.M., 2004. Varicella-zoster virus transfer to skin by T Cells and modulation of viral replication by epidermal cell interferon-alpha. J. Exp. Med. 200, 917–925.

Liu, L., Eby, M.T., Rathore, N., Sinha, S.K., Kumar, A., Chaudhary, P.M., 2002. The human herpes virus 8-encoded viral FLICE inhibitory protein physically associates with and persistently activates the Ikappa B kinase complex. J. Biol. Chem. 277, 13745–13751.

Luftig, M., Prinarakis, E., Yasui, T., Tsichritzis, T., Cahir-McFarland, E., Inoue, J., Nakano, H., Mak, T.W., Yeh, W.C., Li, X., Akira, S., Suzuki, N., Suzuki, S., Mosialos, G., Kieff, E., 2003. Epstein-Barr virus latent membrane protein 1 activation of NF-kappaB through IRAK1 and TRAF6. Proc. Natl. Acad. Sci. U.S.A. 100, 15595–15600.

Mallory, S., Sommer, M., Arvin, A.M., 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.

Massague, J., 1998. TGF-beta signal transduction. Annu. Rev. Biochem. 67, 753–791.

Matta, H., Chaudhary, P.M., 2004. Activation of alternative NF-kappa B pathway by human herpes virus 8-encoded Fas-associated death domain-like IL-1 beta-converting enzyme inhibitory protein (vFLIP). Proc. Natl. Acad. Sci. U.S.A. 101, 9399–9404.

Mayne, M., Cheadle, C., Soldan, S.S., Cermelli, C., Yamano, Y., Akhyani, N., Nagel, J.E., Taub, D.D., Becker, K.G., Jacobson, S., 2001. Gene expression profile of herpesvirus-infected T cells obtained using immunomicroarrays: induction of proinflammatory mechanisms. J. Virol. 75, 11641–11650.

Mo, C., Lee, J., Sommer, M., Grose, C., Arvin, A.M., 2002. The requirement of varicella zoster virus glycoprotein E (gE) for viral replication and effects of glycoprotein I on gE in melanoma cells. Virology 304, 176–186.

Moffat, J., Ito, H., Sommer, M., Taylor, S., Arvin, A.M., 2002. Glycoprotein I of varicella-zoster virus is required for viral replication in skin and T cells. J. Virol. 76, 8468–8471.

Moses, A.V., Jarvis, M.A., Raggo, C., Bell, Y.C., Ruhl, R., Luukkonen, B.G., Griffith, D.J., Wait, C.L., Druker, B.J., Heinrich, M.C., Nelson, J.A., Fruh, K., 2002. Kaposi's sarcoma-associated herpesvirus-induced upregulation of the c-kit proto-oncogene, as identified by gene expression profiling, is essential for the transformation of endothelial cells. J. Virol. 76, 8383–8399.

Mossman, K.L., Macgregor, P.F., Rozmus, J.J., Goryachev, A.B., Edwards, A.M., Smiley, J.R., 2001. Herpes simplex virus triggers and then disarms a host antiviral response. J. Virol. 75, 750–758.

Pahl, H.L., 1999. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18, 6853–6866.

Parikh, A.A., Salzman, A.L., Kane, C.D., Fischer, J.E., Hasselgren, P.O., 1997. IL-6 production in human intestinal epithelial cells following stimulation with IL-1 beta is associated with activation of the transcription factor NF-kappa B. J. Surg. Res. 69, 139–144.

Patel, A., Hanson, J., McLean, T.I., Olgiate, J., Hilton, M., Miller, W.E., Bachenheimer, S.L., 1998. Herpes simplex type 1 induction of persistent NF-kappa B nuclear translocation increases the efficiency of virus replication. Virology 247, 212–222.

Paulose-Murphy, M., Ha, N.K., Xiang, C., Chen, Y., Gillim, L., Yarchoan, R., Meltzer, P., Bittner, M., Trent, J., Zeichner, S., 2001. Transcription program of human herpesvirus 8 (kaposi's sarcoma-associated herpesvirus). J. Virol. 75, 4843–4853.

Pierelli, L., Marone, M., Bonanno, G., Mozzetti, S., Rutella, S., Morosetti, R., Rumi, C., Mancuso, S., Leone, G., Scambia, G., 2000. Modulation of bcl-2 and p27 in human primitive proliferating hematopoietic progenitors by autocrine TGF-beta1 is a cell cycle-independent effect and influences their hematopoietic potential. Blood 95, 3001–3009.

Ray, N., Enquist, L.W., 2004. Transcriptional response of a common permissive cell type to infection by two diverse alphaherpesviruses. J. Virol. 78, 3489–3501.

Roberts, A.B., 1998. Molecular and cell biology of TGF-beta. Miner. Electrolyte Metab. 24, 111–119.

Ruzinova, M.B., Benezra, R., 2003. Id proteins in development, cell cycle and cancer. Trends Cell Biol. 13, 410–418.

Sato, B., Ito, H., Hinchliffe, S., Sommer, M.H., Zerboni, L., Arvin, A.M., 2003. Mutational analysis of open reading frames 62 and 71, encoding the varicella-zoster virus immediate-early transactivating protein, IE62, and effects on replication in vitro and in skin xenografts in the SCID-hu mouse in vivo. J. Virol. 77, 5607–5620.

Sato, H., Callanan, L.D., Pesnicak, L., Krogmann, T., Cohen, J.I., 2002. Varicella-zoster virus (VZV) ORF17 protein induces RNA cleavage and is critical for replication of VZV at 37 degrees C but not 33 degrees C. J. Virol. 76, 11012–11023.

Sherlock, G., Hernandez-Boussard, T., Kasarskis, A., Binkley, G., Matese, J.C., Dwight, S.S., Kaloper, M., Weng, S., Jin, H., Ball, C.A., Eisen, M.B., Spellman, P.T., Brown, P.O., Botstein, D., Cherry, J.M., 2001. The Stanford Microarray Database. Nucleic Acids Res. 29, 152–155.

Sommer, M.H., Zagha, E., Serrano, O.K., Ku, C.C., Zerboni, L., Baiker, A., Santos, R., Spengler, M., Lynch, J., Grose, C., Ruyechan, W., Hay, J., Arvin, A.M., 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.

Stevenson, D., Xue, M., Hay, J., Ruyechan, W.T., 1996. Phosphorylation and nuclear localization of the varicella-zoster virus gene 63 protein. J. Virol. 70, 658–662.

Stingley, S.W., Ramirez, J.J., Aguilar, S.A., Simmen, K., Sandri-Goldin, R.M., Ghazal, P., Wagner, E.K., 2000. Global analysis of herpes simplex virus type 1 transcription using an oligonucleotide-based DNA microarray. J. Virol. 74, 9916–9927.

Taddeo, B., Esclatine, A., Roizman, B., 2002. The patterns of accumulation of cellular RNAs in cells infected with a wild-type and a mutant herpes simplex virus 1 lacking the virion host shutoff gene. Proc. Natl. Acad. Sci. U.S.A. 99, 17031–17036.

Taddeo, B., Luo, T.R., Zhang, W., Roizman, B., 2003. Activation of NF-kappaB in cells productively infected with HSV-1 depends on activated protein kinase R and plays no apparent role in blocking apoptosis. Proc. Natl. Acad. Sci. U.S.A. 100, 12408–12413.

Tsavachidou, D., Podrzucki, W., Seykora, J., Berger, S.L., 2001. Gene array analysis reveals changes in peripheral nervous system gene expression following stimuli that result in reactivation of latent herpes simplex virus type 1: induction of transcription factor Bcl-3. J. Virol. 75, 9909–9917.

Tusher, V.G., Tibshirani, R., Chu, G., 2001. Significance analysis of microarrays applied to the ionizing radiation response. Proc. Natl. Acad. Sci. U.S.A. 98, 5116–5121.

Verrecchia, F., Mauviel, A., 2002. Transforming growth factor-beta signaling through the Smad pathway: role in extracellular matrix gene expression and regulation. J. Invest. Dermatol. 118, 211–215.

Wang, Z.H., Gershon, M.D., Lungu, O., Zhu, Z., Mallory, S., Arvin, A.M., Gershon, A.A., 2001. Essential role played by the C-terminal domain of glycoprotein I in envelopment of varicella-zoster virus in the trans-Golgi network: interactions of glycoproteins with tegument. J. Virol. 75, 323–340.