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Original article

Microarray analysis of transcriptional responses to infection by herpes simplex virus types 1 and 2 and their US3-deficient mutants

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Abstract

Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) induce similar responses in infected cells and animals but differ in several significant respects. Previous studies have shown that defects in the US3-encoded protein kinase greatly affect both viruses in their interactions with cells and hosts. To investigate the impact of infection with HSV-1, HSV-2 and their US3-deficient mutants (ΔUS3) on cellular transcriptional responses, we performed a global microarray analysis on human epithelial HEp-2 cells that were mock-infected, or infected with wild-type (WT) HSV-1, HSV-2 and their ΔUS3 mutants. Among 54,765 probe sets examined, only 1156 (approximately 2.1%) and 2006 (approximately 3.7%) genes increased by at least fourfold at 9 h postinfection in WT HSV-1 and HSV-2-infected cells, respectively. Unexpectedly, HSV-2 infection increases mRNA levels for a larger number of cellular genes than HSV-1 infection. Additionally, ΔUS3 infection upregulated the expression of a larger number of cellular genes than WT infection. The genes affected by HSV infection were assigned to various groups of functional classes and cellular pathways. We have thus identified cellular genes whose expression was similarly or differently changed by infection with each virus.
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Keywords: Herpes simplex virus type 1; Herpes simplex virus type 2; US3; Microarray; Cellular transcriptional response

1. Introduction

Interactions between viruses and host cells are complex, multifaceted processes. While viruses attempt to take over cellular functions for their advantage, cells counteract by mounting a variety of defense responses, including innate immune responses, apoptotic pathways, signal transduction pathways, transcription and protein metabolism, all of which involve changes in gene expression [1]. The recent development of microarray technology has allowed a comprehensive analysis of transcriptional changes in cells, and the technology has proven useful to analyze transcriptional responses to viral infections [2].

Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) are large, enveloped DNA viruses with a 152 kilobase pair (bp) genome. HSV-1, the Alphaherpesvirinae prototype, is the most intensively studied herpesvirus. HSV-1 infection activates cellular signal transduction pathways, including the NF-κB pathway [3], p38 mitogen-activated protein kinase and Jun N-terminal kinase (JNK) cascades [4,5], and modulates the apoptotic response of host cells [6,7]. HSV-2 has almost an identical set of genes and induces similar responses in most infected cell types [8–10]. However, HSV-2 significantly differs from HSV-1 in several biological properties [11], and the molecular mechanism for these differences remains unclear.

The *US3* genes of HSV-1 and HSV-2 encode a serine/threonine protein kinase [12–14], and US3 homologs are

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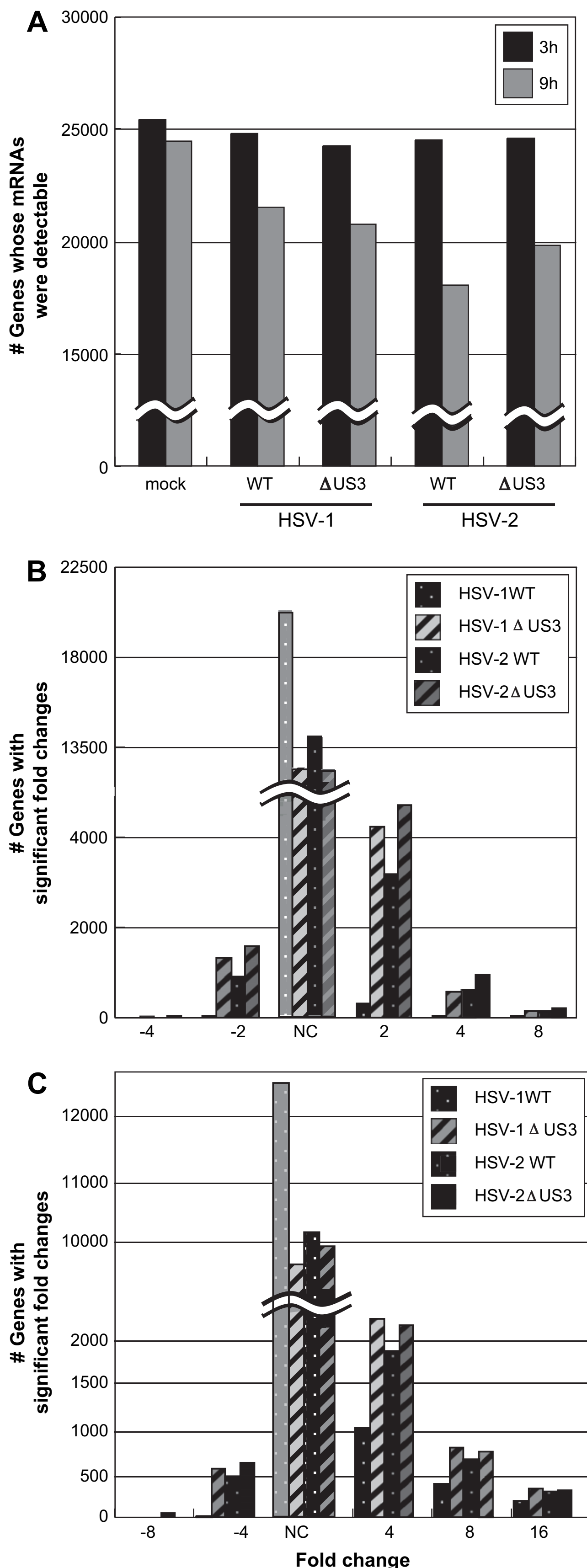

Fig. 1. (A) Number of cellular genes that were judged to be "present" by DNA microarray viewer. (B) and (C) The graph compares the distribution of fold changes in gene expression between HSV-1 WT (F), HSV-1 ΔUS3 (R7041), HSV-2 WT (186), or HSV-2 ΔUS3 (L1BR1)-infected cells and mock-infected cells. Each category includes all genes that have at least the fold change indicated on the *X*-axis. NC, no change; B, 3 h postinfection; C, 9 h postinfection.

conserved in all alphaherpesviruses, including varicella-zoster virus (VZV), and pseudorabies virus (PRV) [13]. While not essential for virus growth *in vitro* [14], a US3 PK deficiency has profound effects on viral pathogenicity [15]. US3 PK is multi-functional and has been implicated in the nuclear egress of viral capsids [16], prevention of apoptosis [6,7,9], and alteration of the actin cytoskeleton [10]. Moreover, US3 PK contributes to interferon resistance, suppresses antigen-presenting cell activation [17] and inhibits cytotoxic T lymphocyte effector functions [18]. Our previous study showed that a HSV-2 US3-deficient mutant (ΔUS3) enhanced immune responses despite severe attenuation, suggesting that defects in US3 may be a promising strategy to develop genital herpes vaccines [17]. Thus, the roles of US3 PK in viral replication and virus–host interactions are of great interest.

In this study, we analyzed the transcriptional responses of human epithelial HEp-2 cells infected with WT HSV-1 and HSV-2 and their ΔUS3 mutants using cDNA microarray technology. We found significant changes in the expression of genes associated with a variety of cellular pathways. This study represents the first global analysis of cellular transcriptional responses to HSV-2 infection.

2. Materials and methods

2.1. Cells, viruses, and infection

Vero cells, a stable African monkey kidney cell line, were propagated in Eagle's minimal essential medium (MEM) containing 10% calf serum. Human HEp-2 cells were propagated in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum (FCS). All viruses were propagated and titered on Vero cells. The HSV-1 WT strain F and its ΔUS3 mutant R7041 were kindly provided by B. Roizman, University of Chicago. The properties of HSV-2 WT strain 186 and its ΔUS3 mutant L1BR1 were described previously [8,9,15,17,19].

Confluent HEp-2 cells plated in 10-cm dishes were mock-infected or infected at three plaque-forming units (PFU) of HSV-1 (F, R7041) or HSV-2 (186, L1BR1) per cell for 60 min at 37 °C, followed by the addition of DMEM containing 1% FCS.

2.2. RNA isolation

At the indicated times postviral infection, the medium was removed and cells were washed with PBS and then lysed with ISOGEN (Nippon Gene Co., Toyama, Japan). Lysates were stored at −80 °C. RNA for microarray analysis was isolated

with ISOGEN and purified with the RNeasy MiniElute kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. RNA quality was assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Double-stranded cDNA was synthesized using a T7-oligo (dT) primer with the One-cycle cDNA synthesis kit (Affymetrix Inc., Santa Clara, CA) and subsequently purified using a Sample Cleanup Module (Affymetrix).

2.3. Microarray data analysis

Hybridization samples were prepared and processed according to the GeneChip Expression Analysis Technical Manual, 701021 Rev. 5. The Human Genome U133 plus 2.0 chips comprise 54,765 probe sets and provide comprehensive coverage of the transcribed human genome on a single array, allowing the analysis of more than 47,000 transcripts and variants, including 38,500 well-characterized human genes plus approximately 6500 new genes. Data were analyzed using the GeneChip Operating Software version 1.4 (Affymetrix 690036) according to the GeneChip Expression Analysis Data Analysis Fundamentals, Part No. 701190 Rev. 4.

Using DNA MicroArray Viewer (Kurabo, Osaka, Japan) fold changes in expression between each of the infected samples compared to mock-infected controls of the same cell type were calculated, log 2 transformed, and further classified as not changed, increased (signal log ratio change P-value of <0.005), decreased (signal log ratio change P-value of >0.995), or marginally increased or decreased. Sequences that showed differential expression in infected cells were grouped according to the GeneOntology terms for biological processes, which were available on the National Center for Biotechnology Information website (March 2006, NCBI Build 36.1). Sequences not yet annotated by GeneOntology were not analyzed further. Genes that could be placed into more than one group according to these annotations were arbitrarily assigned to a single group.

2.4. Microarray data validation by real-time PCR

PCR primers and TaqMan MGB probes (FAM dye-labelled) for the following genes were products from TaqManR gene expression assays (Applied Biosystem, Forester City, CA): IL-8 (Hs00174103_m1), TUBB2B (Hs00603550_g1), DUSP6 (Hs00169257_m1), FOS (Hs00170630_m1), GADD45B (Hs00169587_m1), and GAPDH (Hs99999905_m1). Reactions were set-up in 20 µl volumes with fivefold cDNA dilutions, 20× TaqMan gene expression assay mix, and TaqMan Universal PCR master mix (Applied Biosystem). PCRs were performed in duplicate following the manufacture's protocols on an model Mx3000P real-time PCR system (Stratagene, La Jolla, CA) using the following protocol: an initial denaturation and polymerase activation step for 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and 60 °C for 1 min. GAPDH was used as a reference gene to normalize between samples.

3. Results

3.1. Overall cellular gene expression during HSV infection

To identify cellular genes whose expression levels change during HSV infection, human epithelial HEp-2 cells were mock-infected or infected with WT HSV-1 or HSV-2 or their ΔUS3 mutants. We chose this cell line because HEp-2 cells are widely used as a permissive human cell line in experimental HSV studies. When HEp-2 cells were infected with HSV, viral DNA synthesis was yet at undetectable levels at 3 h postinfection, but at 9 h postinfection the true late genes whose synthesis is strictly dependent on viral DNA replication were fully expressed. We thus examined cellular gene expression at 3 h and 9 h postinfection.

Total RNA was extracted from mock-infected and infected cells, and subjected to microarray analysis using the GeneChip Affymetrix U133 plus 2.0 of 54,765 probe sets representing approximately 47,000 human transcripts. Among these transcripts, 25,416 (46.40%) at 3 h postinfection and 24,443 (44.63%) at 9 h postinfection were detectable in mock-infected cells after hybridization with probes prepared from uninfected HEp-2 cells, while approximately 44.3–45.3% and 33.0–39.4% were detectable at 3 h and 9 h postinfection in HSV-infected cells, respectively. The overall number of cellular genes with increased expression was greater in ΔUS3-infected cells than in WT-infected cells for both HSV-1 and HSV-2 (Fig. 1).

These genes were assembled in Venn diagrams to display differently and similarly regulated genes between WT- and ΔUS3-infected cells. The Venn diagrams included genes that were increased at least fourfold. As shown in Fig. 2, the number of cellular genes affected by HSV infection increased as infection progressed. Among the 54,765 probe sets examined, only 1156 (approximately 2.1%) and 2006 (approximately 3.7%) genes increased by at least fourfold at 9 h postinfection in WT HSV-1- and HSV-2-infected cells, respectively.

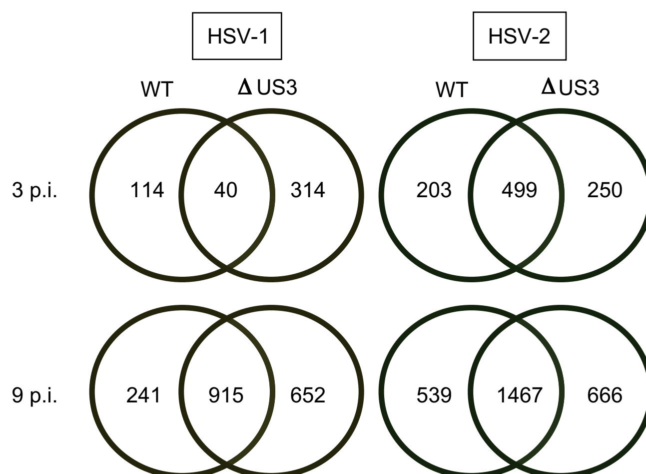

Fig. 2. Venn diagrams of changed genes in HSV-1 and HSV-2-infected cells. Genes that changed fourfold compared to mock-infected cells were sorted into Venn diagrams. The number of upregulated genes that fit the selection criteria is shown.

3.2. Cellular genes affected by HSV infection

We grouped cellular genes according to the GeneOntology annotation of biological processes. Genes were classified into 12 groups, and the majority of genes fell into functional categories that are known or likely to be important in host responses to viral infections, including immune responses, apoptosis, signal transduction, cell adhesion and transcription factors. Table 1 shows the distribution of the number of cellular genes whose expression was increased at least fourfold by HSV at 9 h postinfection. In this GeneOntology annotation, most genes were classified into two groups. Upregulated genes were observed in every group of biological processes, and the number of upregulated genes in ΔUS3-infected cells was greater in all groups than that in WT-infected cells. The data also showed that the number of cellular genes was similarly upregulated by infection with either ΔUS3 strain.

Genes whose expression was significantly affected at 3 h (Supplementary Table S1) and 9 h postinfection (Table 2) were assigned to the selected 12 groups. The complete list of upregulated or downregulated genes at 3 h and 9 h postinfection is provided in the Supplementary material (http://www.med.nagoya-u.ac.jp/virus/kamakura.html).

Transcritpion of some genes involved in the immune response was increased by HSV infection. IFN alpha/beta play a critical role in defense against HSV infection, and IFN-regulatory factors (IRF), especially *IRF-1*, *IRF-3* and *IRF-7*, are important transcriptional activators of IFN alpha/beta during viral infection. At 3 h postinfection, *IRF-1* gene expression increased in HSV-infected cells (Supplementary Table S2). At 9 h postinfection, *IRF-1* levels further increased, and the expression of *IRF-4* and *IRF-7* genes also increased in HSV-infected cells. The expression of *IFN alpha* and *IFN beta* genes was undetectable in HSV-infected HEp-2 cells at 3 h and 9 h postinfection. However, HSV infection increased mRNA levels of antiviral genes such as 2,5-oligoadenylate synthetase1 (*OAS1*).

Chemokines are an important functional subgroup of IFN inducible cytokines, and *CXCL3* (GRO gamma or MIP2 beta) at 3 h postinfection and *CXCL2* (GRO beta or MIP2 alpha) at 9 h postinfection had increased gene expression in HSV-infected cells. Upregulation of *CCL3* gene expression was observed only in HSV-2 WT-infected cells. *IL-8* gene expression was strongly induced 3 h postinfection, and upregulation continued at least until 9 h postinfection. Increased *IL-11* gene expression was observed only in ΔUS3-infected cells at 3 h postinfection (Supplementary Table S2). At 3 h postinfection, *SOCS2* gene expression significantly increased. In addition, the expression of *SOCS4*, *SOCS5*, and *SOCS6* genes was also upregulated.

We also analyzed the transcriptional profiles of several apoptosis-related genes. The transcriptional level of *BIRC3* (hIAP1) increased during HSV infection at 3 h postinfection (Supplementary Table S2). In contrast, the expression of other anti-apoptotic genes, *FAIM* and *BIRC5* (survivin), was downregulated by HSV infection (Supplementary Table S3). Among Bcl-2 family members, the expression of pro-apoptotic genes, *BCL2L11* (BIM) and *PMAIP1* (NOXA), was upregulated. However, the transcripts of anti-apoptotic genes such as *BCL-2*, *BCL2L1* (Bcl-xL), and *BCL2L2* (Bcl-w) except for *MCL1* were undetectable in both mock-infected and HSV-infected cells. *MCL1* was approximately two- and fourfold upregulated (Supplementary Table S2). The expression of caspase family genes was weakly up- or downregulated (approximately between 1.4- and 1.9-fold, data not shown). *PPP1R15A* (GADD34) mRNA levels increased in HSV-infected cells at 9 h postinfection.

HSV infection affected a number of genes associated with signal transduction that regulate a variety of physiological responses. The transcriptional expression of *MAP3K5* (ASK1), *MAP3K8* and *MAPK1* (ERK2) was upregulated during HSV infection. We also found that dual specific phosphatases, such as *DUSP1*, *DUSP4*, and *DUSP6*, were upregulated as early as 3 h postinfection (Supplementary Table S2), and *DUSP4* and *DUSP6* levels were further increased at 9 h postinfection. Thus observations suggest that the expression of both positive and negative regulators of MAPK signaling increased during HSV infection.

The expression of cell adhesion-related genes, such as *ITGA6*, *CDH5* and *CDH15* at 3 h postinfection (Supplementary Table S2) and *PCDH7*, was upregulated in HSV-2 infected cells. At 9 h postinfection, the level of *DLL1*, a Notch signaling-related gene, was strongly increased. The expression of cytoskeleton-related genes, such as *TUBB2A* and *TUBB2B*, was also markedly increased. However, *AROC5*, *PALLD*, and *HMMR* were downregulated by HSV infection (Supplementary Table S3).

For DNA/chromosome-related genes, some histone family members such as *HIST2H2AA3*, *HISTIH2BG*, and *HISTIH2AM* were upregulated during HSV infection, while *H2AFY*, a member of the H2A histone family, was downregulated.

The largest group of cellular genes affected by HSV infection was associated with transcriptional regulation. The levels of transcription factor *FOS* increased at 3 h postinfection (Supplementary Table S2), and a marked increase was observed at 9 h postinfection for all HSV infections. *JUN* at 3 h postinfection (Supplementary Table S2), *JUNB*, *EGR1*, and *NFKBIA* at 9 h postinfection, which are induced in response to diverse signals, were also upregulated by HSV infection. Moreover, some zinc finger proteins, including *ZNF595*, *ZNF342*, and *ZSCAN4*, were markedly upregulated by HSV infection. Increases in *ZNF236* gene expression were observed only in HSV-2 ΔUS3-infected cells.

The expression of RNA metabolism- and translation-related genes, such as *EIF4A2* and *MKNK2*, was induced by HSV infection. In addition, *NXF1*, *ZFP36*, and *PABPC1* were strongly upregulated by infection with any HSV, while the expression of *AUH*, an AU RNA-binding protein, was increased only in ΔUS3 infections. The enhanced expression of *EIF2AK3* (PERK) was observed as early as 3 h postinfection (Supplementary Table S2).

Among genes involved in protein folding, modification, and degradation, proteasome–ubiquitin-related genes were both upregulated and downregulated. The expression of ubiquitin-specific

Table 1
Categories of human genes dispalying altered transcription profiles during HSV infection of HEp-2 cells at 9 h postinfection

GeneOntology biological process and GenBank accession no.[a]	Gene symbol	Gene title	Fold change[b]			
			HSV-1		HSV-2	
			WT	ΔUS3	WT	ΔUS3
Up regulation						
Immune response						
M57731	*CXCL2*	Chemokine (C-X-C motif) ligand 2	5.7	4.3	9.8	9.8
AF134715	*TNFSF13B*	Tumor necrosis factor (ligand) superfamily, member 13b	13.0	18.4	7.0	9.8
NM_000584	*IL-8*	Interleukin 8	16.0	7.0	32.0	26.0
NM_002983	*CCL3*	Chemokine (C-C motif) ligand 3	–	–	68.6	–
NM_002463	*MX2*	Myxovirus (influenza virus) resistance 2	–	–	–	4.6
Apoptosis						
U83981	*PPP1R15A*	Protein phosphatase 1, regulatory (inhibitor) subunit 15A	4.6	6.1	3.0	3.2
NM_021127	*PMAIP1*	Phorbol-12-myristate-13-acetate-induced protein 1	4.3	5.3	5.7	6.1
AF021233	*TNFRSF10D*	Tumor necrosis factor receptor superfamily, member 10d, decoy with truncated death domain	17.1	22.6	13.0	13.9
AI760495	*CYCS*	Cytochrome *c*, somatic	8.6	12.1	12.1	13.0
NM_006538	*BCL2L11*	BCL2-like 11 (apoptosis facilitator)	9.8	14.9	14.9	16.0
NM_001924	*GADD45A*	Growth arrest and DNA-damage-inducible, alpha	10.6	13.0	16.0	13.9
NM_015675	*GADD45B*	Growth arrest and DNA-damage-inducible, beta	22.6	27.9	17.1	19.7
NM_003897	*IER3*	Immediate early response 3	21.1	22.6	27.9	26.0
Cell cycle						
NM_019084	*CCNJ*	Cyclin J	4.3	5.7	5.7	4.3
NM_001432	*EREG*	Epiregulin	6.5	5.3	13.0	12.1
AY137580	*CDC25A*	Cell division cycle 25A	6.5	14.9	9.2	12.1
AY034790	*CCNL1*	Cyclin L1	21.1	55.7	24.3	32.0
NM_003672	*CDC14A*	CDC14 cell division cycle 14 homolog A (*S. cerevisiae*)	–	5.3	–	4.3
Signal transduction						
NM_005923	*MAP3K5*	Mitogen-activated protein kinase kinase kinase 5	2.3	4.3	–	2.8
NM_013254	*TBK1*	TANK-binding kinase 1	2.0	3.5	2.6	2.8
NM_005204	*MAP3K8*	Mitogen-activated protein kinase kinase kinase 8	5.3	3.7	4.9	3.7
NM_138957	*MAPK1*	Mitogen-activated protein kinase 1	2.3	3.5	4.6	6.1
BC002671	*DUSP4*	Dual specificity phosphatase 4	29.9	42.2	104.0	97.0
BC003143	*DUSP6*	Dual specificity phosphatase 6	34.3	64.0	32.0	45.3
AF069506	*RASD1*	RAS, dexamethasone-induced 1	48.5	39.4	128.0	36.8
M60278	*HBEGF*	Heparin-binding EGF-like growth factor	73.5	111.4	157.6	147.0
AF178983	*RBJ*	Ras-associated protein Rap 1	–	4.9	–	8.0
AJ302584	*OR2B2*	Olfactory receptor, family 2, subfamily B, member 2	–	19.7	–	26.0
U62027	*C3AR1*	Complement component 3a receptor 1	–	–	–	29.9
Cell adhesion and cytoskeleton						
AK022326	*CTNNA1*	Catenin (cadherin-associated protein), alpha 1102 kDa	7.0	8.0	2.7	2.6
AA521145	*PLXNC1*	Plexin C1	5.7	8.0	3.0	8.0
U64317	*NEDD9*	Neural precursor cell expressed, developmentally downregulated 9	7.5	5.7	5.7	8.0
AB006756	*PCDH7*	BH-protocadherin (brain–heart)	5.3	7.0	3.7	8.6
BC005253	*KLHL20*	Kelch-like 20 (*Drosophila*)	45.3	73.5	29.9	27.9
NM 001069	*TUBB2A*	Tubulin, beta 2A	21.1	26.0	39.4	34.3
AF196571	*DLL1*	Delta-like 1 (*Drosophila*)	78.8	97.0	119.4	137.2
AL533838	*TUBB2B*	Tubulin, beta 2B	181.0	315.2	222.9	362.0
BC016998	*FAT*	FAT tumor suppressor homolog 1 (*Drosophila*)	–	–	11.3	10.6
NM_030932	*DIAPH3*	Diaphanous homolog 3 (*Drosophila*)	–	–	59.7	64.0
AI700685	*ABLIM2*	Actin binding LIM protein family, member 2	–	4.6	–	–
AW293517	*ABLIM2*	Actin binding LIM protein family, member 2	–	–	–	5.7
NM_006159	*NELL2*	NEL-like 2 (chicken)	–	–	–	5.7
AF498927	*ARGHGDIB*	Rho GDP dissociation inhibitor (GDI) beta	–	–	–	16.0
DNA/chromosome						
NM_000270	*NP*	Nucleoside phosphorylase	4.3	8.0	3.2	4.3
NM_002105	*H2AFX*	H2A histone family, member X	4.6	6.5	6.1	6.1
AL713694	*POLE*	Polymerase (DNA directed), epsilon	6.5	10.6	9.2	11.3
BC017833	*PRIM2A*	Primase, polypeptide 2A, 58 kDa	8.6	24.3	9.2	16.0

(*continued on next page*)

Table 1 (*continued*)

GeneOntology biological process and GenBank accession no.[a]	Gene symbol	Gene title	Fold change[b]			
			HSV-1		HSV-2	
			WT	ΔUS3	WT	ΔUS3
NM_003655	*CBX4*	Chromobox homolog 4 (Pc class homolog, *Drosophila*)	11.3	14.9	14.9	16.0
NM_003516	*HIST2H2AA3*	Histone 2, H2aa3	34.3	36.8	32.0	42.2
BC001131	*HIST1H2BG*	Histone 1, H2bg	16.0	10.6	59.7	52.0
NM_003514	*HIST1H2AM*	Histone 1, H2am	16.0	19.7	36.8	55.7
NM_020135	*WRNIP1*	Werner helicase interacting protein 1	–	–	9.8	9.2
NM_005321	*HIST1H1E*	Histone 1, Hle	–	4.3	–	–
NM_003529	*HIST1H3A*	Histone 1, H3a	–	–	–	4.9
Transcription						
NM_002198	*IRF-1*	Interferon regulatory factor 1	4.9	5.3	6.5	4.3
NM_004030	*IRF-7*	Interferon regulatory factor 7	4.3	4.6	MI[c]	4.3
AI078167	*NFKBIA*	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	6.1	7.0	7.5	7.5
NM_001674	*ATF3*	Activating transcription factor 3	7.5	5.3	7.5	4.6
AF044076	*ING1*	Inhibitor of growth family, member 1	6.5	10.6	4.6	6.5
NM_001964	*EGR1*	Early growth response 1	13.9	18.4	9.2	8.0
U58658	*TP53*	Tumor protein p53 (Li–Fraumeni syndrome)	5.7	8.0	7.5	13.9
NM_002229	*JUNB*	Jun B proto-oncogene	13.9	18.4	18.4	22.6
NM_002166	*ID2*	Inhibitor of DNA binding 2, dominant negative helix–loop–helix protein	17.1	19.7	27.9	26.0
BC004490	*FOS*	v-fos FBJ murine osteosarcoma viral oncogene homolog	45.3	52.0	64.0	55.7
AI580142	*ZNF595*	Zinc finger protein 595	84.4	147.0	157.6	207.9
NM_152677	*ZSCAN4*	Zinc finger and SCAN domain containing 4	388.0	1782.9	168.9	274.4
AA761573	*ZNF342*	Zinc finger protein 342	274.4	445.7	831.7	1024.0
NM_002460	*IRF-4*	Interferon regulatory factor 4	–	14.9	14.9	13.9
NM_002357	*MXD1*	MAX dimerization protein	–	6.1	–	8.0
BE675108	*KLF12*	Kruppel-like factor 12	–	9.8	–	16.0
BQ775325	*ST18*	Suppression of tumorigenicity 18 (breast carcinoma) (zinc finger protein)	–	18.4	–	26.0
NM_002196	*INSM1*	Insulinoma-associated 1	–	84.4	–	39.4
NM_002432	*MNDA*	Myeloid cell nuclear differentiation antigen	–	–	–	52.0
AK000847	*ZNF236*	Zinc finger protein 236	–	–	–	1351.2
RNA/translation						
NM_001967	*EIF4A2*	Eukaryotic translation initiation factor 4A, isoform 2	4.0	5.3	4.9	5.3
NM_017572	*MKNK2*	MAP kinase interacting serine/threonine kinase 2	6.1	4.6	6.5	5.3
BC004904	*NXF1*	Nuclear RNA export factor 1	9.8	17.1	11.3	10.6
NM_003407	*ZFP36*	Zinc finger protein 36, C3H type, homolog (mouse)	36.8	34.3	29.9	34.3
R17062	*PABPC1*	Poly(A) binding protein, cytoplasmic 1	59.7	55.7	45.3	48.5
AI791801	*AUH*	AU RNA-binding protein/enoyl-Coenzyme A hydratase	–	13.0	–	11.3
Protein folding and modification						
BC013247	*UBPH*	Ubiquitin-binding protein homolog	4.9	13.9	5.7	14.9
AA021559	*USP36*	Ubiquitin-specific peptidase 36	17.1	22.6	9.8	13.0
AL136825	*USP44*	Ubiquitin-specific peptidase 44	39.4	55.7	64.0	64.0
AF086225	*ZC3HC1*	Zinc finger, C3CH-type containing 1	–	42.2	–	42.2
Transport						
NM_145206	*VTI1A*	Vesicle transport through interaction with t-SNAREs homolog 1A (yeast)	4.3	14.9	9.8	6.5
AL110169	*NUP214*	Nucleoporin 214 kDa	4.9	9.8	7.0	8.0
AW194689	*ERN1*	Endoplasmic reticulum to nucleus signaling 1	6.1	11.3	13.9	14.9
V00489	*HBA1*	Hemoglobin, alpha 1	90.5	111.4	157.6	207.9
T50399	*HBA2*	Hemoglobin, alpha 2	207.9	512.0	512.0	724.1
Cellular metabolism						
NM_004566	*PFKFB3*	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	6.1	4.3	4.0	4.9
NM_001362	*DIO3*	Deiodinase, iodothyronine, type III	5.7	5.7	8.0	8.0
AK090598	*LCTL*	Lactase-like	12.1	17.1	13.0	7.5
NM_001124	*ADM*	Adrenomedullin	36.8	52.0	48.5	48.5
AL050388	*SOD2*	Superoxide dismutase 2, mitochondrial	36.8	–	90.5	238.9
AI248622	*PCYT1A*	Phospate cytidylyltransferase 1, choline, alpha	–	6.1	–	5.7
NM_024743	*UGT2A3*	UDP glucuronosyltransferase 2 family, polypeptide A3	–	–	–	19.7

Table 1 (*continued*)

GeneOntology biological process and GenBank accession no.[a]	Gene symbol	Gene title	Fold change[b]			
			HSV-1		HSV-2	
			WT	ΔUS3	WT	ΔUS3
Miscellaneous						
AF143875	*KIAA0974*	KIAA0974	18.4	36.8	21.1	27.9
AW025183	*LOC389332*	Hypothetical LOC389332	8.6	–	36.8	–
NM_003323	*TULP2*	Tubby like protein 2	18.4	–	10.6	–
BC022419	*PHTF2*	Putative homeodomain transcription factor 2	–	4.0	–	4.3
BC037938	*LOC389765*	Similar to KIF27C	–	4.9	–	5.3
AI660254	*CCDC92*	Coiled-coil domain containing 92	–	5.7	–	4.6
AV725825	*ZCCHC12*	Zinc finger, CCHC domain containing 12	–	6.5	–	6.5
AL834399	*SPPL3*	Signal peptide peptidase 3	–	11.3	–	8.0
AL049394	*BTBD7*	BTB (POZ) domain containing 7	–	48.5	–	12.1
AI732310	*LOC644010*	Hypothetical protein LOC644009	–	14.9	–	21.1

–, No change or absent.
[a] GenBank accession no. corresponds to sequence to which the Affymetrix U133 plus 2.0 probe set was designed.
[b] Fold change was calculated by comparison to values obtained from the mock-infected cells.
[c] MI, marginal increase.

peptidase (USP) genes, such as *USP36* and *USP44*, was upregulated by HSV infection, while that of ubiquitin conjugating enzyme genes, such as *UBE2D2* and *UBE2 M*, was downregulated at 9 h postinfection. A number of genes encoding proteasome subunits, such as *PSMA8*, *PSMB9* (Supplementary Table S2) and *PSMA4*, were also downregulated in infected cells.

One purpose of this study was to identify specific genes with significant transcriptional differences between WT- and ΔUS3-infected cells. Cellular genes upregulated by ΔUS3 mutants but not by WT HSV-1 and HSV-2 were as follows: *CDC14A*, *RBJ*, *OR2B2*, *MXD1*, *KLF12*, *ST18*, *INSM1*, *AUH*, *ZC3HC1*, and a number of functionally unknown genes (*SPPL3*, *ZCCHC12*, and *BTBD7*). Interestingly, *ZNF236* whose product belongs to the Kruppel family of C2H2-type zinc finger proteins and functions as a glucose-regulated transcriptional factor was strikingly upregulated only in HSV-2 ΔUS3-infected HEp-2 cells.

We further compared HSV-induced alteration in expression of cellular genes between WT HSV-1 and WT HSV-2, WT HSV-1 and its ΔUS3, and WT HSV-2 and its ΔUS3. The results are shown in Table 3 and Supplementary Table S4. Interestingly, HSV-2, compared with HSV-1, induced enhanced expression of a number of cellular genes involved in immune responses and signal transduction as early as 3 h postinfection.

3.3. Confirmation of microarray data for selected genes by real-time RT-PCR

To evaluate the reliability of the expression changes detected by the microarray analysis, we used real-time RT-PCR to analyze five selected genes that represented the entire range of expression changes from 2.5- to 782.0-fold (Table 4). The *GAPDH* transcript was chosen as an internal control since expression of this message was not affected by HSV. The RT-PCR results confirmed the microarray analysis, although the levels of upregulation were quantitatively different between the two methods. Our findings are comparable to those observed in similar microarray analyses of HSV-1- and VZV-induced changes in host gene expression in infected primary human fibroblasts [20,21].

Table 2
Number of up regulated genes for each biological process categories at 9 h postinfection

GeneOntology biological process	No. of genes (%)	HSV-1		HSV-2		Common	ΔUS3 specific
		WT	ΔUS3	WT	ΔUS3		
Immune response	1229 (2.25)	17	32	40	42	13	1
Apoptosis	1642 (3)	55	99	78	99	40	2
Cell cycle	1908 (3.49)	50	105	88	97	34	4
Signal transduction	5349 (9.78)	95	195	192	213	56	7
Cell adhesion and cytoskeleton	2268 (4.14)	23	73	61	73	10	5
DNA/chromosome	940 (1.72)	40	63	56	67	26	0
Transcription	6235 (11.4)	151	358	264	332	93	8
RNA/translation	3089 (5.65)	94	209	172	200	65	3
Translation	579 (1.06)	14	40	29	35	10	2
Protein folding and modification	1370 (2.51)	23	59	53	57	17	1
Transport	5739 (10.5)	73	167	120	159	40	3
Cellular metabolism	5728 (10.48)	96	192	166	199	50	4
Unknown	23,720 (43.3)	395	940	777	892	211	22

Genes were placed into more than one group according to GeneOntology term for biological processes.

Table 3
Number of genes modified by each HSV-infected in HEp-2 cells

Change (-fold)	3 h			9 h		
	HSV-2/HSV-1[a]	ΔUS3/HSV-1 WT[b]	ΔUS3/HSV-2 WT[c]	HSV-2/HSV-1[a]	ΔUS3/HSV-1 WT[b]	ΔUS3/HSV-2 WT[c]
−8	0	0	0	0	1	0
−4	0	1	0	90	20	0
−2	449	555	1	1176	991	28
2	1630	1320	86	907	783	98
4	132	173	22	137	72	22
8	55	45	12	61	38	14
16	18	25	5	26	18	4

[a] 186, Compared to F-infected HEp-2 cells.
[b] R7041, compared to F-infected HEp-2 cells.
[c] L1BR1, compared to 186-infected HEp-2 cells.

4. Discussion

HSV-1 and HSV-2 cause very similar responses in infected cells, but they affect cellular functions differently. For example, HSV-2 upregulates while HSV-1 downregulates nitric oxide production in epithelial cells [22]. Although both types of HSV inhibit cellular mRNA and protein synthesis early during infection of permissive cells, HSV-2 generally causes faster and stronger shut-off than HSV-1 [23]. The present study demonstrates that HSV-2 infection increases mRNA levels for a larger number of cellular genes than HSV-1 although the overall number of downregulated genes was significantly larger in HSV-2-infected cells than in HSV-1-infected cells. In addition, the magnitude of downregulation was greater in HSV-2-infected cells. These observations suggest that biological differences between HSV-1 and HSV-2 can partly be explained by these different transcriptional responses.

The nuclear factor NF-κB is a key regulator of cellular events [24]. NF-κB-binding sites have been identified in the promoter region of more than 300 cellular genes. Functionally important NF-κB-binding sites are also located in the genome of several viruses, including members of the herpesvirus family [3]. It was recently reported that HSV infection rapidly activates the NF-κB activation pathway characterized by the association and subsequent translocation of NF-κBp65 (RelA) to the nucleus [3]. In this study, we found that several genes reported to be NF-κB targets were upregulated at the transcriptional level, including *IL-6*, *IL-8*, *IL-11*, *CCL3*, *IRF-1*, *IRF-4*, *TNFAIP3*, *PTGS*, *SOD2*, *NFKB1A*, etc. [20,21,25]. Among these NF-κB target genes, *IL-6*, *IL-8*, *IL-11*, *F3*, and *CCL3* are immune response-related genes, *IRF-1*, *IRF-4*, and *IRF-7* are transcriptional factors, *TNFAIP3* (A20) acts in signal transduction pathways, and *PTGS2* and *SOD2* are involved in cellular stress responses. The upregulation of these genes could play critical roles in HSV–host cell interactions.

We have identified many cellular genes whose expression specifically increased in cells infected with the ΔUS3 mutants. The significance of the upregulation of those genes is not yet understood, but increased AUH expression may stabilize mRNA in ΔUS3-infected cells since the product of AUH, an AU RNA-binding protein, stabilizes early response genes such as transcription factors. The product of ZNF236 belongs to the Kruppel family of C2H2-type zinc finger proteins and functions as a glucose-regulated transcriptional factor. We found that *ZNF236* expression strikingly increased during the early phase of infection only in HSV-2 ΔUS3-infected cells. At 9 h postinfection, mRNA levels reached approximately 1350-fold compared to that in mock-infected cells. This dramatic change in mRNA levels may provide a clue for solving differences between HSV-1 and HSV-2.

In summary, we report that different subsets of genes are upregulated at different time points following HSV infection, reflecting the impact of the virus on cell metabolism and the subsequent defense responses of the host cell. Since microarrays are semiquantitative analyses, differences in mRNA levels between infected and non-infected cells reported here

Table 4
Validation of array data by real-time PCR[a]

Gene	Change (fold) determined at 9 h postinfection							
	Microarray data for				Real-time PCR for			
	HSV-1		HSV-2		HSV-1		HSV-2	
	WT	ΔUS3	WT	ΔUS3	WT	ΔUS3	WT	ΔUS3
IL-8	16	7	32	26	17.9	16.4	40.7	58.7
DUSP6	34.3	64	32	45.3	4.4	4.2	2.5	3.5
FOS	45.3	52	64	55.7	19	16.8	10.3	13.7
GADD45B	17.1	24.3	13.9	17.1	16	15.3	17.5	18.6
TUBB2B	181	315.2	222.9	362	461	782	324	510

[a] Real-time PCR conditions are described in Section 2.

should not be taken as absolute figures [26]. Nevertheless, the large amount of data generated by this microarray provides interesting clues to many processes in HSV-infected cells and opens new lines of study and investigation.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.micinf.2007.12.019.

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