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Transcriptional Response of a Common Permissive Cell Type to Infection by Two Diverse Alphaherpesviruses

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Pseudorabies virus (PRV) and herpes simplex virus type 1 (HSV-1) are distantly related alphaherpesviruses whose natural hosts are pigs and humans, respectively. Adult infections of natural hosts are mild and rarely lethal. However, both viruses are also able to infect other hosts, often with lethal effects. In this report, we use the paradigm of infection of a common permissive cell type and microarray analysis to determine if these two diverse alphaherpesviruses engage similar or different cellular pathways to obtain a common outcome: productive infection. We compared cellular gene expression in growth-arrested, primary rat embryonic fibroblasts that were mock infected or infected with either purified PRV-Becker or HSV-1(F). Infections by either virus affect the transcription of more than 1,500 cellular genes by threefold or more. Few differences are detected early, and the majority of changes occur during the late stages of infection. Remarkably, the transcripts of about 500 genes are regulated in common, while the rest are regulated in a virus-specific manner. Genes whose expression is affected by infection fall into a diverse group of functional classes and cellular pathways. Furthermore, a comparison of the cellular response to HSV-1 infection of primary human and rat fibroblasts revealed unexpected diversity in the transcript profiles.

Pseudorabies virus (PRV) and herpes simplex virus type 1 (HSV-1) are distantly related members of the subfamily *Alphaherpesvirinae*. PRV belongs to the *Varicellovirus* genus, whereas HSV-1 belongs to the *Simplexvirus* genus of alphaherpesviruses; by sequence comparison, the two viruses are placed at distal branches of an alphaherpesvirus phylogenetic tree (35). By direct DNA-DNA hybridization, only 8% of the sequences of their genomes is homologous (7). Nevertheless, PRV and HSV-1 genes share many positional and functional homologues (29), and their replication strategies are remarkably similar (36). PRV and HSV-1 can infect some cells, such as rat fibroblasts and neurons, by using the same cellular receptors for entry (e.g., the HveC receptor) (40).

The natural hosts for PRV and HSV-1 are pigs (domestic or feral) and humans, respectively. Adult infections of natural hosts are mild and rarely lethal. HSV-1 cannot infect pigs and PRV cannot infect humans (38, 49, 50, 60), but both viruses can infect other hosts, often with lethal effects. For example, HSV-1 and PRV can infect and kill most rodents such as mice, rats, and hamsters (2, 9, 17–19, 22, 34, 41, 59). In these nonnatural hosts, HSV-1 and PRV invade the peripheral nervous system from the site of infection and spread into the central nervous system. This property has led neuroanatomists to exploit PRV as a tracer for mapping networks of synaptically connected neurons in rats and other species (reviewed in reference 16).

Rodent infection models have long been used to study the pathogenesis promoted by both viruses. PRV mutants attenuated in the natural host also exhibit reduced virulence in rodents, a finding supporting the validity of the approach (5, 6, 54). Similar observations have also been documented for HSV-1 infections (reviewed in reference 16). These common virulence phenotypes and pathogenic outcomes in diverse hosts such as pigs, humans, mice, and rats may reflect common molecular interfaces of host and viral gene products during infection. To test this hypothesis, we used microarray technology to determine if two diverse alphaherpesviruses engage similar or different cellular pathways during the productive infection of a common permissive cell type. In particular, we used primary cultures of rat cells that are equally susceptible and permissive to infection by either virus.

Considerable work using HSV-1 infection of cultured human or monkey cells to study the cellular response has been done. HSV-1 infection can modulate the apoptosis response (reviewed in reference 3) and has been found to activate NF-κB pathway via IκB kinase activation (1), the Jun N-terminal kinase/stress-activated protein kinase, and p38 mitogen-activated protein kinase (MAPK) cascades (61). In addition, HSV-1 genes modulate host gene expression by posttranscriptional mechanisms, altering mRNA stability, mRNA transport, and translation (reviewed in reference 46). For PRV, mRNA differential display has been used to evaluate the cellular response to infection (23); however, the cellular pathways affected by PRV infection have not been studied in detail.

In recent years, microarray technology has proven useful for assessing the cellular transcriptional response to viral infections (10, 24, 37, 51, 53). In the present study, we examined the cellular mRNA levels at several times after PRV or HSV-1 infection of primary cultures of rat embryonic fibroblasts (REF). Using Affymetrix microarrays, we identified genes whose expression increased or decreased more than threefold over that of the mock-infected group upon infection. We then annotated these genes, classified them into functional groups, and subsequently assigned a subset of these to cellular pathways. We found that most host transcriptional changes occurred late after infection by both viruses and that both common and virus-specific pathways could be identified. Additionally, com-

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parison of data obtained from HSV-1 infection of REF cells and primary human fibroblasts indicated a diverse host-specific cellular response.

MATERIALS AND METHODS

Viruses and cells. In order to obtain high virus yields, PRV-Becker (PRV-Be) and HSV-1 strain F [HSV-1(F)] stocks were grown on PK15 and Vero cells, respectively. For virus purification, typically 8×10^7 PK15 or Vero cells were infected at a multiplicity of infection of 10 PFU per cell with PRV-Be or HSV-1(F). When complete cytopathic effect was observed, the majority of HSV-1 virions were found to remain cell associated, whereas PRV virions were present in the extracellular medium. Accordingly, we collected either the cells and extracellular media (for HSV-1) or only the extracellular media (for PRV) at the time of complete cytopathic effect. The virus-containing medium was clarified in a clinical centrifuge (Sorvall H-1000B rotor) at 2,200 rpm for 10 min and layered onto a 6-ml 30% sucrose cushion; the virions were then pelleted at 23,500 rpm for 60 min in a Beckman SW28 rotor. The virion pellet was resuspended in TNE (50 mM Tris-HCl, 150 mM NaCl, 10 mM EDTA), layered onto 20 to 50% dipotassium tartrate step gradients (6 ml of 20% tartrate on top of 2.5 ml of 50% tartrate) formed in phosphate-buffered saline (PBS) and centrifuged for 90 min at 24,100 rpm in an SW41 rotor with slow braking. The virions formed a band at the interface of the 50 and 20% tartrate phases. The virion band was pulled by using a 22-gauge needle, diluted three- to fourfold with PAE (PBS with 2 μg of aprotinin per ml and 1 mM EDTA), and pelleted by centrifugation at 20,500 rpm for 60 min with an SW41 rotor. The virion pellet was layered onto a linear 10-ml 20 to 50% dipotassium tartrate gradient formed in PBS and spun at 24,100 rpm for 16 h in an SW41 rotor. The virions separated into a tight lower band (infectious virions) and a diffuse upper band (presumably L particles). The lower band was pulled, and virions were pelleted as described before. The virion pellet was resuspended in 150 μl of PAE and separated into aliquots for titration and Western blot analysis. No immature forms of PRV or HSV-1 glycoproteins were detected in our virion preparations by Western blotting, a finding attesting to their purity (data not shown).

REF cells were isolated from gestation day 13 rat embryonic tissue, expanded, and frozen using standard protocols by C. Paulus (21). Passage 12 cells were used for all experiments. Cells were grown to confluence in Dulbecco's modified Eagle's medium (supplemented with 10% fetal bovine serum) at 37°C in a 5% CO_2 atmosphere in a humidified chamber; the cells were then growth arrested by maintaining in the same (spent) medium for at least 10 days postconfluence.

Growth-arrested cells were infected with purified virions diluted in spent medium to a multiplicity of infection of 5 PFU/cell or with an equivalent volume of PAE buffer diluted in spent medium (for the mock-infected group). After 1 h of adsorption at 37°C, the inocula were replaced with warm spent medium. At the indicated times after the addition of virus, the medium was removed, the cells were lysed with TRIzol (Invitrogen), and the lysates were stored at −80°C. For each time point, three independent infections were carried out.

Cell viability of infected, growth-arrested REF cells was tested by using the Cell Titer Aqueous One solution cell proliferation assay (Promega) and following the manufacturer's protocol.

RNA purification and microarray hybridization. Total RNA was isolated from TRIzol lysates according to the manufacturer's instructions. cDNA, derived from 20 μg of total RNA, was used as the template for biotin-labeled cRNA synthesis as described in the Affymetrix technical manual. The labeled cRNA samples were hybridized to Affymetrix RGU34A arrays. For each time point, the cRNA samples from three independent infections were hybridized to three different sets of arrays. The hybridized arrays were stained and washed in GeneChip fluidics stations by using the EukGE-WS2 v4 protocol defined in MAS 5.0 (Affymetrix). The microarrays were scanned with a GeneArray scanner system.

Data analysis. The expression data were subjected to global scaling in MAS 5.0, using a target intensity of 150. The metrics values were imported into GeneSpring (Silicon Genetics) and normalized to the average of the corresponding time-matched mock-infected samples. The data were filtered to exclude probe sets that were absent in all conditions and then to retain only those whose expression varied from the averaged mock value with a maximum *t* test *P* value of 0.05 for at least one condition.

It is important that although the RGU34A arrays contain 8,799 probe sets, a few genes are represented by more than one probe set. These arrays also contain some annotative redundancies (a single gene might have several names or GenBank accession numbers corresponding to it). These redundancies were removed when the genes affected by infection were classified into functional classes.

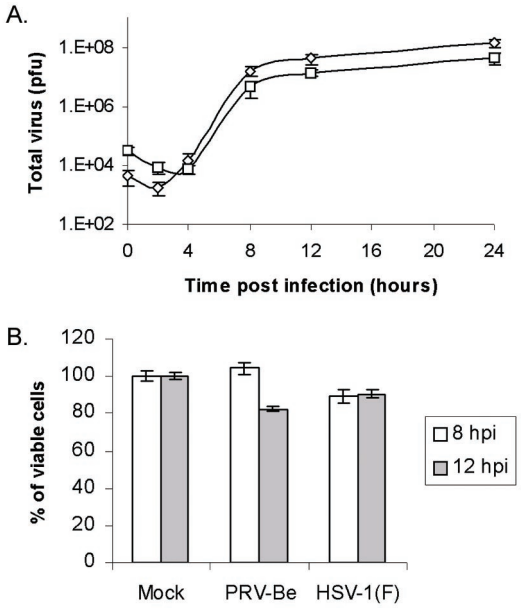

FIG. 1. PRV and HSV-1 display similar growth kinetics in REF cells and keep these cells metabolically active for up to 12 hpi. (A) Single-step growth kinetics of PRV-Be (◇) and HSV-1(F) (□) on growth-arrested REF cells. Scale on the *y* axis is logarithmic ($n = 4$). (B) Determination by colorimetric assay of the number of metabolically active PRV- and HSV-1-infected REF cells at 8 and 12 hpi, expressed as a percentage of mock-infected cells ($n = 3$). The values for mean ± standard error of the mean are plotted.

Real-time PCR. cDNAs corresponding to mock-infected and infected samples were used for real-time PCR analysis; the time point chosen corresponded to that when the gene of interest displayed maximal change of expression in microarrays. Primers were designed by using the PrimerExpress 2.0 software (Applied Biosystems [ABI]). Reactions were set up in a 25-μl volume with 10-fold dilutions of cDNA, PCR primers (100 nM), and SYBR Green PCR master mix (ABI). An ABI PRISM 7900 sequence detection system was used for monitoring the level of SYBR green fluorescence over 40 cycles of PCR. At the end of the cycling phase, a dissociation curve was produced by slow denaturation of the PCR end products to ensure specificity of amplification. For each sample, the quantity of cDNA corresponding to the gene of interest was normalized to the quantity of 18S rRNA. Relative expression levels were calculated by using the $2^{-\Delta\Delta C_t}$ method (33) if the primer pair for the gene of interest passed a validation test described in the ABI user bulletin number 2 (http://docs.appliedbiosystems.com/pebiodocs/04303859.pdf). If the primer pair failed validation, a standard curve method described in the same publication was used.

RESULTS

Infection of growth-arrested REF cells with PRV and HSV-1. The standard method of infecting nonconfluent cells in various stages of the cell cycle failed to yield microarray hybridization data that were sufficiently reproducible (data not shown). Infection of growth-arrested REF cells increased the reproducibility of gene expression data among replicate samples. The quiescent cells were easily infected and produced high yields of infectious PRV or HSV-1 virions (Fig. 1A). Moreover, these cells were remarkably robust: infected cells displayed cytopathic effects (rounding up, nuclear distension) by 6 h postinfection (hpi) but maintained membrane integrity and remained attached to the plate beyond 12 hpi. Viability of REF cells infected with PRV and HSV-1 at 8 and 12 hpi was measured by examining the level of cellular metabolic activity, as determined by the bioreduction of a tetrazolium compound

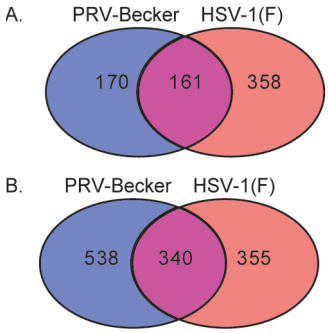

FIG. 2. Distribution of probe sets with more than threefold increased (A) or decreased (B) levels after PRV or HSV-1 infection. Depicted are Venn diagrams showing the number of probe sets that changed after infection by either or both viruses.

into a colored formazan product. As shown in Fig. 1B, the vast majority of cells were still metabolically active and intact 12 h after infection by either virus.

Differential cellular gene expression after PRV and HSV-1 infection. Growth-arrested REF cells were mock infected or infected with PRV-Be or HSV-1(F) purified virions, and total RNA was isolated at 0, 1, 2, 3, 4, 5, 6, 8, and 12 hpi. The experiment was performed in triplicate (samples A, B, and C) for a total of 81 samples. Two of the chips (PRV-5hA and HSV-2hC) were found to have manufacturing defects after the initial scanning and were removed from subsequent analyses. Data from the remaining 79 samples can be found at http://www.herpes.lanl.gov. Expression data were imported into GeneSpring software, normalized, and filtered as described in Materials and Methods.

The filtered data set, consisting of 4,626 probe sets, was then used to deduce differences in gene expression that were effected by infection. Over the 12-h time course, 2,810 probe sets from the filtered data set did not change significantly (i.e., a more than threefold increase or decrease in mRNA levels).

Probe sets whose levels varied threefold or more were selected for further analysis. The distribution of 689 probe sets that increased mRNA levels by more than threefold after PRV or HSV-1 infection is shown in Fig. 2A. PRV and HSV-1 infection increased the mRNA levels of genes corresponding to 331 and 519 probe sets, respectively. Roughly 23% of the 689 total probe sets increased after infection with either virus. Figure 2B shows the distribution of 1,233 probe sets with reduced levels after infection by PRV or HSV-1. PRV and HSV-1 infection decreased the level of 878 and 695 probe sets, respectively. The expression of genes represented by 340 probe sets was reduced after infection with either virus. After removal of annotation- and probe set-related redundancies (see Materials and Methods), we found that the expression of 498 genes was regulated similarly by PRV and HSV-1, while 521 were affected only by PRV infection and 530 were altered only after HSV-1 infection.

Temporal regulation of host gene expression after PRV and HSV-1 infection. Distribution of the number of probe sets affected by PRV and HSV-1 infections at different times postinfection can be seen in Table 1. At early time points (0 to 3 hpi), PRV and HSV-1 infections increased the mRNA levels of 46 genes and decreased those of 83 genes by more than threefold (Table 2). However, the majority of transcriptional changes occurred late, between 8 and 12 hpi (Table 1). A similar study in human foreskin fibroblasts infected with HSV-1(F) found that while expression of 70 genes increased and 64 genes decreased more than threefold at 1 hpi, the majority of changes occurred later (450 genes increased and 310 decreased at 7 hpi) (51).

Independent confirmation of microarray data. We selected 15 genes whose expression was identified in the microarray analysis to be significantly affected by virus infection. Two of these genes (Arc and Btg2) were also tested for both PRV and HSV-1 infections, so that a total of 17 real-time PCR analyses were completed. The genes were chosen to represent a wide range of expression patterns and *n*-fold-change values. Of the 17 microarray data points tested using real-time PCR, 13 (76.5%) verified the array data, while 4 samples were discordant (Table 3). Our findings are comparable to those observed in similar microarray analyses of HSV-1-induced changes in host gene expression in infected primary human fibroblasts (51).

Genes regulated by PRV and HSV-1 infection belong to diverse functional classes. The 1,549 genes whose expression is regulated by PRV and/or HSV-1 infection were classified by hand into 24 functional classes (Table 4). Of the 1,549 genes, 419 were expressed sequence tags (ESTs) and 117 were not easily assigned. The two largest classes of genes were involved in metabolism and signaling, followed by genes involved in cell adhesion, cellular transport (including genes encoding cytoskeletal components), and transcription factors. Several genes involved in stress response, immunity, and apoptosis were also affected by both virus infections. The complete list of functionally classified genes regulated by the two viruses can be found in the supplemental information (available at http://www.molbio.princeton.edu/labs/enquist/SupplInfo.html).

Pathways affected by PRV and HSV-1 infection. One indication that the observed transcript differences may have biological relevance is if sets of genes in known pathways show coordinated regulation. Accordingly, the functionally classified genes listed in the supplemental information were mapped to known cellular pathways. Table 5 shows a list of genes that were assigned to these cellular pathways, and some of these are discussed in detail below.

TGF-β signaling. The expression of many genes belonging to the transforming growth factor β (TGF-β) signaling path-

TABLE 1. Number of probe sets affected by PRV-Be and HSV-1(F) infection

Time (hpi)	No. of probe sets affected by infection with:			
	PRV[a]	HSV-1[a]	PRV[b]	HSV-1[b]
0	8	11	12	22
1	7	7	23	12
2	7	6	4	16
3	6	6	19	6
4	12	10	1	8
5	30	22	10	4
6	33	6	22	7
8	49	11	19	35
12	251	489	794	613

[a] Threefold (or more) increase in mRNA levels.
[b] Threefold (or more) decrease in mRNA levels.

TABLE 2. Genes regulated early (0 to 3 hpi) by PRV and/or HSV-1 infection

Gene		Data for PRV		Data for HSV	
GenBank no.	Description	Maximum change (*n*-fold)	Time (h)	Maximum change (*n*-fold)	Time (h)
Threefold (or more) increase (0–3 hpi)					
AA892854	ESTs, small inducible cytokine B13 precursor	3.74	0	—[a]	—[a]
AA892986	EST	3.66	0	—	—
AI175900	Ets1	3.41	0	—	—
AB008521	Dynein light intermediate chain 53/55	3.37	0	—	—
L26110	TGF-β type I	3.02	0	—	—
AA800851	Carboxylesterase 3	4.48	1	—	—
X07266	Gene 33 polypeptide	3.72, 4.23	1.3	—	—
AA799593	EST	3.72	1	—	—
AA800882	EST	3.59	1	—	—
J04526	Hexokinase M	3.15	1	—	—
M60786	Endothelin receptor	3.51	2	—	—
U63318	TGF-β type 1 receptor	3.15	2	—	—
J00712	Casein gamma	3.03	2	—	—
X06769	c-*fos*	4.19	3	—	—
U78102	Krox20 (NGFI-B or Egr2)	3.27	3	—	—
X13722	LDL receptor	—[a]	—[a]	3.75	0
M17527	GTP-binding protein (G-alpha-i1)	—	—	3.55	0
AI639224	Ribosomal protein S13	—	—	3.39	0
U14647	IL-1β converting enzyme (IL-1βCE)	—	—	3.13	0
AI014135	Beta carotene 15, 15-dioxygenase	—	—	3.03	0
M31032	Contiguous repeat polypeptides (CRP)	—	—	4.24	1
U04937	Na-Ca exchanger isoform NACA1	—	—	3.78	1
M35602	Beta fibrinogen	—	—	3.53	1
AI639444	Sarcosin (sarcomeric muscle protein)	—	—	3.52	1
AA893216	Kidney-specific membrane protein	—	—	3.23	2
M57682	Brain calcium channel alpha-1 subunit	—	—	3.19	2
AI058601	Ankyrin 3 (G), Ank3	—	—	3.18	2
H31479	EST	—	—	4.36	3
AA800693	EST	—	—	3.42	3
Z35138	Fibroblast growth factor receptor 2b	—	—	3.19	3
AF036537	Homocysteine-respondent protein HCYP2	4.93	2	3.05	1
AA955554	Splicing factor, arginine/serine-rich 5	3.74	2	−4.20	1
L26292	Follicle-stimulating hormone-regulated protein (clone 59)	5.38	3	−3.16	2
Z27513	Carbamoylphosphate synthase I	−12.08	1	7.02, −9.43	0, 2
AA875097	EST, fibrinogen alpha/alpha-E chain precursor	−3.16	1	4.28, 3.07	0, 3
AF083331	Kinesin-like protein KIF1B	−4.38	1	3.24	0
AA875123	EST	−5.06	1	3.84	2
AA800456	EST	−4.14, −3.06	1, 2	3.47	3
AA875390	Thioredoxin-like protein	8.29	0	4.48	0
H31859	ESTs	3.91	0	8.13	0
AI175764	Stearoyl-coenzyme A desaturase 1 (Scd1)	3.46, −4.41, −3.11	0, 1, 3	9.32, −3.82	0, 3
AI639318	Ret proto-oncogene	−3.38, 4.06	0, 1	6.45	1
AA800908	EST	3.91	1	4.26	1
AA946542	Prolactin-like protein D	−10.61, 4.33	1, 2	−4.67, 4.31	1, 2
AI639233	Decorin	3.1, −4.23	2, 3	−3.75, 3.15	0, 2
AF030088	Activity and neurotransmitter-induced gene (ania-3)	3.67	3	3.63	3
Threefold (or more) decrease (0–3 hpi)					
U25282	Brush border myosin (BBMI)	−5.50	0	—	—
U59801	Integrin alpha-M (Itgam)	−5.02	0	—	—
M22926	Muscarinic acetylcholine receptor M5	−3.60	0	—	—
M82845	Peptidylglycine alpha-amidating monooxygenase-5	−3.59, −4.28	0, 3	—	—
AI639244	EST	−3.47, −3.29	0, 3	—	—
AI176191	Vomeronasal neurons pheromone receptor V2R2	−3.29	0	—	—
L06804	LIM homeodomain protein (LH-2)	−3.24	0	—	—
AI639125	EST	−3.09	0	—	—
AI639244	Neonatal submandibular gland protein B, Smgb	−7.30	1	—	—
X06107	IGF-I	−6.48, −3.02	1, 2	—	—
Z83757	Growth hormone receptor	−4.86	1	—	—
AF039832	Homeobox protein (rPtx2)	−4.49	1	—	—
AI228110	UDP-glucuronosyltransferase 8 (Ugt8)	−4.08	1	—	—
AI639446	EST	−4.00	1	—	—
L22655	Anti-acetylcholine receptor Ab[b] gene, kappa chain	−3.93	1	—	—
L39018	Sodium channel protein 6 (SCP6)	−3.84	1	—	—
AF034753	DC16 mRNA	−3.57	1	—	—
AA892240	ESTs, 2008109A set gene (weakly similar)	−3.32	1	—	—

Continued on facing page

TABLE 2—*Continued*

Gene		Data for PRV		Data for HSV	
GenBank no.	Description	Maximum change (*n*-fold)	Time (hpi)	Maximum change (*n*-fold)	Time (hpi)
AI176460	EST	−3.14	1	—	—
M86758	Estrogen sulfotransferase	−6.54	3	—	—
M31788	Phosphoglycerate kinase	−6.82	3	—	—
M88709	Cell adhesion-like molecule	−5.48	3	—	—
AA926193	Sulfotransferase family 1C, member 2, Sult1c2	−5.15	3	—	—
D50664	Oligopeptide transporter	−4.82	3	—	—
U32681	Ebnerin	−3.67	3	—	—
AI136175	Rab38	−3.65	3	—	—
D31734	Distal-less 3 (Dlx-3) homeobox protein	−3.63	3	—	—
U95157	Ryanodine receptor type II	−3.30	3	—	—
AI639466	EST	−3.23	3	—	—
M58287	Nonspecific lipid transfer protein (nsL-TP)	−3.12	3	—	—
J05189	Neuromedin K receptor	−3.10	3	—	—
AI639358	EST	−3.08	3	—	—
AI639263	EST	−3.05	3	—	—
AA800930	EST	—	—	−6.67	0
AA799488	EST	—	—	−6.16	0
L24207	Testosterone 6-beta-hydroxylase (CYP3A1)	—	—	−4.44	0
L07315	Dipeptidase (dpep1)	—	—	−4.44	0
AI639315	EST	—	—	−4.43	0
AA799488	Secretogranin II	—	—	−4.40	0
AI030997	Toll-like receptor 4	—	—	−4.36	0
AF043642	Matrin cyclophilin	—	—	−4.04	0
AA799538	ESTs, cold-inducible RNA-binding protein	—	—	−4.03	0
AI639301	EST	—	—	−4.00	0
M64867	Serotonin receptor	—	—	−3.75	0
D38629	Adenomatosis polyposis coli protein	—	—	−3.65	0
M36419	Glutamate receptor (GluR-B)	—	—	−3.64	0
AA818240	Nuclear pore complex protein	—	—	−3.46	0
AF055714	Hypertension-regulated vascular factor-1C-4	—	—	−3.44	0
AA891650	EST	—	—	−3.30	0
AI639155	EST	—	—	−3.25	0
M31837	Insulin-like growth factor binding protein 3, IGF-BP3	—	—	−3.18	0
D84418	Chromosomal protein HMG2	—	—	−3.15	0
AF020046	Integrin alpha OX-62	—	—	−3.01	0
AI639311	EST	—	—	−7.37	1
Z56277	CLC-5 chloride channel	—	—	−4.20	1
AF000901	p23 (p58/p45) mRNA	—	—	−3.85	1
M25892	Murine IL-4	−3.22	0	−3.65, −3.33	1, 2
AI639468	EST	—	—	−3.11	1
U17837	Retinoblastoma-interacting zinc finger protein	—	—	−3.06	1
X03369	Beta-tubulin T beta15	—	—	−7.93	2
AF093267	Homer-1b	—	—	−5.60	2
M60811	LINE 1 repeat element, ORF[c] II	—	—	−4.57	2
M58495	NAD(P)H: quinone reductase	—	—	−4.12	2
AF065433	Bcl-2-related ovarian death gene BOD-L	—	—	−3.86	2
AF087945	Nitzin mRNA	—	—	−3.80	2
AF065431	Bcl-2-related ovarian death gene BOD	—	—	−3.27	2
AA799475	EST	—	—	−3.18	2
L14680	Bcl-2	—	—	−3.15	2
E00444	DNA coding for gamma interferon	—	—	−3.12	2
AA893242	Fatty acid coenzyme A ligase, long chain 2	—	—	−3.10	2
U78517	Cyclic AMP-GEFII	—	—	−3.00	2
J00777	Fat prostatic steroid-binding protein, C3 peptide	—	—	−3.72	3
L27059	Putative phosphodiesterase	—	—	−3.71	3
AA860015	EST	—	—	−3.24	3
AI639355	EST	—	—	−3.12	3
AA893870	EST	—	—	−3.01	3
U10303	Edg-1 orphan receptor	−7.73	0	−3.32	1
M14775	Cytochrome P-450	−3.98	0	−4.60	1
AA892799	ESTs, 3-phosphoglycerate dehydrogenase	−3.68	1	−3.37	1
X51615	Connexin protein Cx26	−4.07	1	−4.18	1
U52950	Microtubule-associated protein 1B	−4.88	2	−4.24	2
D29646	ADP-ribosyl cyclase (CD38)	−4.46	3	−4.30	0

[a] —, no change or absent.
[b] Ab, antibody.
[c] ORF, open reading frame.

TABLE 3. Validation of array data by real-time PCR

Gene for:	Microarray data for:				RT-PCR data for:	
	PRV		HSV		PRV	HSV
	Maximum change (*n*-fold)	Time (hpi)	Maximum change (*n*-fold)	Time (hpi)	Change (*n*-fold)	Change (*n*-fold)
Amd1a	—[a]	—	5.13	12	ND[b]	−1.3
Arc	6.41	6	7.98	12	31.65	4.16
Btg2	4.88	6	4.63	12	6.06	−2.35
Casp-3	−7.88	12	—	—	−1.65	ND
Cox-2	11.21	8	3.41	12	60.7	ND
Egr-1	9.38	8	—	—	13.8	ND
Gene33	41.49	8	—	—	11.1	ND
HO-1	4.06	8	—	—	2	ND
IL-15	−3.83	12	—	—	−10.5	ND
Lnk-1	4.06	8	—	—	7.5	ND
MKP-3	10.92	8	—	—	4	ND
Odc-1	—	—	14.81	12	ND	−1.8
PRG-1	3.18	8	—	—	−1.7	ND
SGK	5.93	8	—	—	15.2	ND
TagE4	3.25	8	—	—	3.7	ND

[a] —, Not changed or absent.
[b] ND, not done.

way was decreased at 12 hpi by PRV infection. These include farnesyltransferase alpha (an insulin target that interacts with the TGF-β receptor [48, 56]), Golgi protein MG-160 (latent TGF-β complex protein 1 [25, 39]), TIEG, Smad1, and TGF-α. HSV-1 infection also resulted in decreased expression of TGF-β signaling genes, such as TGF-β3, TGF-β1, Smad2, follistatin-related protein (upregulated by TGF-β1 [62]), and fibulin 5 (induced by TGF-β [45]). Moreover, genes such as TSC-22, p8 mRNA (a TGF-β-responsive gene that enhances Smad transcriptional activity [20]), and latent TGF-β-BP2 (an extracellular matrix protein that targets TGF-β action [43]) also displayed reduced expression after both infections. In addition, the Kruppel-like transcription factor Zf9/COPEB (induces uPA expression and activates latent TGF-β1 [30]) and PAI-1 (a TGF-β1-responsive gene that detaches cells from the extracellular matrix by inactivating integrins [14]) were induced at 8 hpi by PRV. PRV and HSV-1 infections also increased the expression of the TGF-β type I receptor gene at 12 hpi.

MAPK signaling genes. MAPK signaling genes were expressed to various levels following infection by PRV and HSV-1. PRV infection reduced expression of p38 MAPK at 12 hpi and increased the expression of MKP-1 (oxidative stress-inducible protein tyrosine phosphatase) and heme oxygenase 1 (oxidative stress-inducible via MAPKs of Jun N-terminal kinase and p38 pathways) at 8 hpi. PRV infection also reduced the expression of Elk protein (downstream target of p38 MAPK) and MAPKK2 at 12 hpi. HSV-1 infection reduced expression of MAPKK2, MAPKK, MAPK9, and MAPKKKK4 and increased expression of MEK5 and of the dual-specificity phosphatase (cpg21) at 12 hpi. Zn finger protein (Zfp36/Tis11/TTP) expression increased 12 h after PRV infection and after HSV-1 infection at 8 hpi, whereas MKP-3 (which dephosphorylates ERK1/2 [42]) expression increased 8 h after PRV infection and decreased 12 h after infection by either PRV or HSV-1. Infection by either virus reduced the levels of MAPK6 at 12 hpi.

JAK/STAT pathway. JAK/STAT pathway genes such as JAK1, STAT3, the p32 subunit of replication protein A (which regulates STAT3 transcription [26]) and SH-2 pleckstrin homology containing signaling mediator 1 (JAK2 substrate) were expressed to lower levels 12 h after infection by PRV. HSV-1 infection increased expression of *neu* differentiation factor (i.e., neuregulin, which activates the JAK/STAT pathway [32]) at 12 hpi. STAT5B expression was also increased by both infections at 12 hpi.

IFN- and IL-1-related pathways. Interferon (IFN)- and interleukin 1 (IL-1)-related genes were regulated primarily by HSV-1 but not by PRV infection. Expression of IFN regulatory factor 3 and IFN-induced mRNA was reduced, while that of IFN-β, IFN regulatory factor 1, IFN-related developmental regulator 1, and IFN consensus sequence binding protein was induced at 12 hpi by HSV-1 infection. However, HSV-1 and PRV infections decreased expression of the IFN-γ gene at 2 and 6 hpi, respectively. IL-1β-converting enzyme expression increased early and decreased late after HSV-1 infection. Soluble IL-1 receptor type 1 expression was reduced, while that of IL-1 receptor accessory protein and IL-1β propeptide was induced at 12 hpi by HSV-1 infection.

IGF pathway. The insulin-like growth factor (IGF) pathway was affected by PRV and HSV-1 infections. Infection by either virus results in increased expression of IGF-II and of the acid-labile subunit of the IGF-binding protein (IGF-BP) complex as well as decreased expression of IGF-BP5 at 12 hpi. The IGF-II receptor, IGF-BP3, and the BRL-3A binding protein were expressed to lower levels, whereas IGF-BP2 and IGF-BP were expressed to higher levels 12 h following infection by HSV-1.

TABLE 4. Classes of genes regulated by PRV-Be and HSV-1(F) infection

Functional class	No. of genes	PRV only	HSV only	PRV and HSV
Signaling	146	47	58	41
Metabolism	138	46	49	43
Cell adhesion	96	11	38	47
Cellular transport	85	37	20	28
Transcription factor	55	28	12	15
Neuronal	53	16	22	15
Stress response	49	21	15	13
Channels and transporters	42	7	19	16
Immunity	41	9	17	15
Kinase	38	15	10	13
Protein folding	36	15	14	7
Nucleus and nuclear matrix	34	13	14	7
Ribosomal proteins	25	20	2	3
Oncogenesis	24	10	5	9
Hormone related	22	5	5	12
Apoptosis	20	8	8	4
RNA-binding proteins	18	9	3	6
Cell cycle related	17	4	7	6
Cytokine or chemokine	15	6	7	2
Electron transport	21	5	9	7
Growth factor	12	0	7	5
Prostaglandin related	12	2	5	5
Peroxisome related	8	3	3	2
Translation	6	2	2	2
Unclassified	117	24	42	51
ESTs	419	158	137	124
Total	1,549	521	530	498

TABLE 5. Pathways of genes regulated upon infection with PRV-Be and HSV-1(F)

Gene		Data for PRV		Data for HSV	
GenBank no.	Description	Maximum change (*n*-fold)	Time (hpi)	Maximum change (*n*-fold)	Time (hpi)
TGF-β					
M81225	Famesyltransferase alpha subunit	−7.96	12	—[a]	—[a]
U08136	Golgi protein MG-160	−6.98	12	—	—
AI172476	TIEG, TGF-β-inducible early growth response	−5.89	12	—	—
AF067727	Smad1 protein	−3.38	12	—	—
M31076	TGF-α	−3.29	12	—	—
M24067	PAI-1, plasminogen activator inhibitor 1	6.22	8	—	—
AF001417	Kruppel-like transcription factor Zf9/COPEB	4.27	8	—	—
S98336	Mullerian inhibiting substance	4.37	12	—	—
AA875033	Fibulin 5	—[a]	—[a]	−9.26	12
U03491	TGF-β3	—	—	−8.40	12
X52498	TGF-β1	—	—	−6.79	12
AB017912	Smad2 protein	—	—	−4.63	12
AA849769	Follistatin-related protein	—	—	−3.94	12
L25785	TSC-22, TGF-β-stimulated clone 22	−12.66	12	−6.14	12
Y12760	Latent TGF-β binding protein 2	−10.1	12	−31.25	12
AF014503	p8 mRNA	−6.49	12	−6.99	12
L26110	TGF-β type I receptor	8.87	12	6.94	12
MAPK					
U73142	p38 MAPK	−4.98	12	—	—
S81478	MKP-1/CL 100	3.14, −3.58	8, 12	—	—
J02722	HO-1, heme oxygenase 1	4.06	8	—	—
X13411	Elk protein	3.47	12	—	—
L04485	MAPKK	—	—	−5.63	12
AI231354	MAPK9	—	—	−3.69	12
AI007614	ESTs, MAPKKKK-4 (highly similar)	—	—	−3.39	12
U37462	MAP kinase/ERK kinase 5 (MEK5)	—	—	3.83	12
AF013144	Dual-specificity phosphatase, cpg21	—	—	3.64	12
AA963674	MAPKK2	3.21	12	−8.38	12
X94185	Dual-specificity phosphatase (MKP-3)	10.92, −3.46	8, 12	−3.04	12
AA800613	Zfp36/Tis11	5.69, −6.85	8, 12	4.69	12
M64301	MAPK6 (ERK3)	−6.37	12	−3.83	12
JAK/STAT					
AJ000556	JAK1	−6.04	12	—	—
U57391	SH2-B PH containing signal mediator 1	−5.76	12	—	—
X91810	STAT3	−5.75	12	—	—
X98490	p32 subunit of replication protein A	−3.24	12	—	—
U02320	Neu differentiation factor	—	—	5.65	12
X91988	STAT5B	4.05	12	5.97	12
Insulin-like growth factor					
U59809	IGF-II receptor	—	—	−14.51	12
M91595	IGF binding protein 2	—	—	−5.50	12
A09811	BRL-3A binding protein (IGF-BP)	—	—	−5.12	12
M31837	IGF binding protein 3	—	—	8.17	12
AA924289	IGF binding protein	—	—	3.77	12
X06107	IGF-1	−6.48, −3.02, 3.58, 3.43	1, 2, 8, 12	−5.69	12
AI029920	IGF binding protein 5	−9.09	12	−3.06	12
X17012	IGF-II	4.1	12	13.08	12
S46785	IGF-BP complex acid-labile subunit	3.11	12	3.42	12
PI-3K/Akt					
J05210	ATP citrate-lyase	−6.96	12	—	—
Y15748	PDK1	−6.87	12	—	—
L13193	Brain factor 3 (HFH-BF-3)	3.27	8	—	—
D30040	RAC protein kinase alpha (Akt)	—	—	−6.45	12
S55223	14-3-3 protein beta	—	—	−5.08	12
U01146	Nuclear orphan receptor HZF-3	—	—	6.36	12
L13201	HNF-3/forkhead homologue 1 (HFH-1)	—	—	5.22	12
U50412	PI3K regulatory subunit, p85 alpha	—	—	5.08	12
M87634	Brain factor 1 (HFH-BF-1)	—	—	4.37	12
AJ006710	Phosphatidylinositol 3-kinase (PI3K)	−8.13	12	−5.68	12
AA942751	14-3-3 protein, theta polypeptide	−7.63	12	−4.24	12
AI105076	Murine thymoma viral (v-*akt*) oncogene 2	−4.1	12	−3.06	12
Z46614	Caveolin	−3.55	12	−10.75	12
A1180424	14-3-3 protein, zeta polypeptide	−3.35	12	−6.49	12
L01624	SGK, serum-glucocorticoid-regulated kinase	5.93	8	8.21	12

Continued on following page

TABLE 5—*Continued*

Gene		Data for PRV		Data for HSV	
GenBank no.	Description	Maximum change (*n*-fold)	Time (hpi)	Maximum change (*n*-fold)	Time (hpi)
Apoptosis: Bcl-2 family					
U49930	Caspase 3	−7.88	12	—	—
AF025670	Caspase 6	−4.71	12	—	—
L14680	Bcl-2	—	—	−3.15	2
AA818072	BAD, Bcl-2-associated death agonist	—	—	−12.16	12
AF065433	BimL	−3.48	5	−3.86, 5.48	2, 12
AF065431	BimL	−3.09	6	7.56	12
U49729	rBax alpha	−4.7	12	−5.35	12
X94185	Dual-specificity phosphatase (MKP-3)	10.92, −3.46	8, 12	−3.04	12
Notch signaling					
L38483	Jagged-1 (Notch ligand)	−21.13	12	—	—
D13417	Transcriptional repressor HES-1	−8.15	12	—	—
AF009329	SHARP-1	−4.84	12	—	—
X99267	Presenilin-2	−3.25	12	—	—
AI101320	Jagged-2, Jag2	4.45	12	3.15	12
Insulin					
AB017596	Plasma cell membrane glycoprotein, PC1	−8.75	12	—	—
M81225	Farnesyltransferase alpha subunit	−7.96	12	—	—
X58375	Insulin receptor (IRS-1)	−3.89	12	—	—
U38481	ROK-alpha	−3.02	12	—	—
M64711	Endothelin-1	9.13	12	4.97	12
Endothelial differentiation					
U10699	Edg5	−4.2	12	—	—
AA848831	Edg2	−3.72	8	—	—
AA956930	Endothelin-converting enzyme 1	—	—	4.54	12
U93306	VEGF receptor-2/FLK-1	—	—	3.46	12
AA850734	VEGF	—	—	3.03	12
U10303	Edg-1 orphan receptor	−7.73	0	−3.32	1
AA891746	ESTs, endothelial differentiation factor 1	−3.37	12	−4.98	12
L20913	VEGF-3	−3.18	12	3.95	12
M60786	Endothelin receptor	3.51, −4.72	2, 12	−4.2	12
M64711	Endothelin-1	9.13	12	4.97	12
IL-1					
U14647	IL-1β converting enzyme	—	—	3.13, −3.92	0, 8
U14010	Soluble IL-1 receptor type I	—	—	−3.25	12
U48592	IL-1 receptor accessory protein	—	—	7.39	12
M98820	IL-1β propeptide (active form)	—	—	5.04	12
Interferon related					
AA893384	ESTs, IFN regulatory factor 3 (IRF-3)	—	—	−6.76	12
X61381	IFN-induced mRNA	—	—	−3.67	12
M34253	IFN regulatory factor 1 (IRF-1)	—	—	10.68	12
AI014163	IFN-related development regulator 1	—	—	6.64	12
D87919	IFN-β	—	—	3.59	12
AA892259	ESTs, IFN consensus sequence-BP	—	—	3.5	12
E00444	DNA coding for IFN-γ	−3.8	6	−3.12	2
Prostaglandin synthesis					
U38376	Cytosolic phospholipase A2	−3.12	12	—	—
U28966	Prostacyclin receptor	3.74	12	—	—
S87522	Leukotriene A4 hydrolase	—	—	−8.46	12
AF051895	Lipocortin V	—	—	−4.07	12
AI171962	Annexin 1 (p35) (Lipocortin 1)	—	—	−3.54	12
AI170268	Prostaglandin F receptor	—	—	−3.2	12
AI169372	Arachidonic acid epoxygenase	—	—	7.32	12
AI175764	Scd1	3.46, −4.41, −3.11, −4.9	0, 1, 3, 8	9.32, −3.82, 4.05, −3.29	0, 3, 8, 12
AF036761	Scd2	−10.53	12	−7.04	12
AI145502	Prostaglandin F2 receptor-negative regulator	−4	12	−20	12
AF000901	p23 (cytosolic PG-E synthase)	3.6, −5.66	8, 12	−3.85	1
S67722	Cox-2	11.21	8	3.92	12
Heat shock					
AI104388	Hsp27	−9.1	12	—	—
M11942	Hsc70	−4.97	12	—	—
AA108277	Hsp-E7I	−3.62	12	—	—
AI170613	Hsp10-1	—	—	−3.60	12
Z75029	Hsp70.2	—	—	31.02	12

Continued on facing page

TABLE 5—*Continued*

Gene		Data for PRV		Data for HSV	
GenBank no.	Description	Maximum change (*n*-fold)	Time (hpi)	Maximum change (*n*-fold)	Time (hpi)
Z27118	Hsp70	—	—	26.34	12
X15705	Hst70	−3.67	12	3.68, 7.27	8, 12
AA818604	Hsp 70-1	−3.07	12	23.12	12
AA875620	Hsp70-3	4	12	3.54	12
M14050	Hsp70-5	−18.87	12	−3.91	12
AI176546	ESTs, Hsp90-β	3.12	12	—	—
Oxidative stress					
AI014169	Vdup1	−7.49	12	—	—
AI138143	GST-theta2	−5.08	12	—	—
AI233261	Glutamate-cysteine ligase, regulatory	−4.45	12	—	—
AI136977	FK506 binding protein (BP) 4	−3.39	12	—	—
Z24721	Superoxide dismutase 3	−3.36	12	—	—
J02722	HO-1, heme oxygenase 1	4.06	8	—	—
S81478	MKP-1/CL100	3.14	8	—	—
K01932	GST-Yc	—	—	−8.37	12
AI010083	Peroxiredoxin 1	—	—	−3.25	12
AI235747	GST-alpha	—	—	7.06	12
AA926149	Catalase	—	—	3.93	12
M62642	Hemopexin	—	—	3.78	12
AF058787	HO-3, heme oxygenase 3	—	—	3.58	12
AA875390	Thioredoxin-like protein	8.29	0	4.48, 3.9, −3.13, 4.95	0, 5, 6, 12
AA799650	Peroxiredoxin 3	−3.6	12	−4.33	12
AI228738	FK506-binding protein (BP) 1 (12 kDa)	−6.76	12	−3.04	12
Fibrinolysis					
M23697	Tissue plasminogen activator (t-PA)	−9.41	12	—	—
M24067	PAI-1, Plasminogen activator inhibitor 1	6.22	8	—	—
X71898	uPAR1, Urinary plasminogen activator receptor	5.25	8	—	—
AF001417	Kruppel-like transcription factor Zf9/COPEB	4.27	8	—	—
M35602	Beta fibrinogen			3.53, −3.41	1, 8
M81642	G-protein coupled thrombin receptor	—	—	−5.69	12
M26247	Factor IX			3.48	12
X05861	Gamma fibrinogen			3.22	12
AA875097	EST, fibrinogen alpha	−3.16, 8.7	1, 5	5.08	5
X53565	TGN38 (upregulates PAI-1 secretion)	−16.95	12	−5.21	12

[a] —, no change or absent.

The expression of IGF-I was reduced early after PRV infection but increased late (8 and 12 hpi). HSV-1 infection caused a reduction in IGF-I expression at 12 hpi.

PI3K/Akt survival pathway. Expression of genes in the PI3K/Akt survival pathway was reduced by PRV and HSV-1 infection. In both infections, the mRNA levels of phosphatidylinositol 3-kinase (PI3K), 14-3-3-theta and -zeta (protein kinase C regulatory proteins; PI3K target), murine thymoma viral (v-*akt*) oncogene homolog 2, and caveolin (which induces the PI3K/Akt signaling pathway [47]) were found to be decreased at 12 hpi. In addition, PRV infection decreased expression of ATP citrate-lyase (Akt substrate [8]) and PDK1 (which phosphorylates Akt and is inhibited by binding 14-3-3 [44]), while HSV-1 infection reduced expression of RAC protein kinase alpha (Akt) and 14-3-3-beta at 12 hpi. Transcript levels of SGK (a target of PI3K that phosphorylates forkhead transcription factor FOXO3a [12]) and forkhead transcription factors such as brain factor 3, HZF-3, and HFH-1 increased late after infection by either virus.

Apoptotic pathway. Genes belonging to the Bcl-2 family of pro- and antiapoptotic proteins were altered upon PRV and HSV-1 infection. Caspase 3 and caspase 6 mRNA levels were decreased after PRV infection, whereas infection with HSV-1 reduced the expression of Bcl-2 (antiapoptotic) and Bad (a Bcl-2-associated death agonist). rBax alpha (proapoptotic) mRNA levels were reduced after infection with either virus. BimL (proapoptotic) levels were reduced by PRV infection and induced by HSV-1 infection, and MKP-3 (which causes apoptosis by degradation of Bcl-2) mRNA levels increased after PRV infection at 8 hpi and decreased at 12 hpi after infection by either PRV or HSV-1.

Notch signaling pathway. Notch signaling pathway genes such as jagged-1 (notch ligand [58]), transcriptional repressor HES-1 (notch effector), SHARP-1 (a component of the histone deacetylase corepressor that represses notch target genes in the absence of activated Notch [4]), and presenilin 2 (a component of gamma-secretase that cleaves notch and APP [27, 28]) decreased 12 h after infection by PRV. Infection by either virus increased expression of jagged-2 at 12 hpi.

Heat shock and oxidative stress pathways. Infection by both viruses affected the expression of genes stimulated by heat shock and oxidative stress late after infection. Expression of Hsp27, Hsc70, and Hsp-E71 was reduced, and Hsp90-beta expression was induced by PRV infection. Infection by HSV-1 decreased levels of Hsp10-1 expression but increased those of Hsp70 and Hsp70-2. Infection by both viruses increased

Hsp70-3 and decreased Hsp70-5 expression. However, HSV-1 infection induced the expression of Hsp70-1 and Hst70, while PRV infection repressed these genes. Both viruses reduced expression of the oxidative stress-related genes peroxiredoxin 3 and FK506-BP1. Expression of Vdup1 (which is suppressed by H_2O_2-induced oxidative stress [57]), glutamate-cysteine ligase (a key enzyme in glutathione synthesis), glutathione *S*-transferase (GST)-theta2 (glutathione synthesis), FK506-BP4, and superoxide dismutase 3 was decreased at 12 hpi, while that of the oxidative stress-inducible genes heme oxygenase 1 and MKP-1 was increased at 8 hpi by PRV infection. Infection by HSV-1 reduced mRNA levels of GST-Yc and peroxiredoxin and induced those of GST-alpha, catalase (marker of oxidative stress), hemopexin (an antioxidant that binds and transports heme to prevent heme-mediated oxidative stress [55]), and heme oxygenase 3.

DISCUSSION

We used a comparative virology approach to guide our analysis of the cellular response to infection. The rationale was that despite their low sequence similarity and distinct natural hosts, PRV and HSV-1 display remarkable similarities in their genome structure, gene conservation, virion structure, and replication cycle. Remarkably, of all the cellular genes significantly affected by infection in our study, only 32% (498 out of 1,549) are common to both viruses (Table 4). Moreover, the diversity of gene functions that are regulated after infection by both viruses was unexpected. For most functional classes, the numbers of PRV-specific and HSV-1-specific genes were about equal. In other cases, a functional class was affected upon infection by one virus but not the other. For example, the cellular transport-cytoskeleton class, transcription factors, stress response genes, ribosomal proteins, oncogenesis, and RNA binding protein are more affected by PRV infection than by HSV-1 infection. Similarly, HSV-1 infection affects expression of genes in the cell adhesion, immunity, channels and transporters, and growth factor classes more than does PRV infection. Such variations in the number of PRV- and HSV-1-specific genes belonging to the different classes are not predictable.

After classifying genes into functional groups, we assigned them to various cellular pathways (Table 5). This classification revealed that the transcription of several genes belonging to the PI3K/Akt signaling and other related pathways changed after infection by PRV and HSV-1. Trophic factors (such as IGF-I) have been shown to play a role in promoting cell survival via the PI3K/c-Akt pathway. Phospholipids generated by PI3K act by multiple mechanisms that cooperate to regulate Akt kinase activity (and also that of SGK and some protein kinase C isoforms). One mechanism involves phospholipid binding to Akt, resulting in its relocalization to the plasma membrane, bringing Akt in proximity to regulatory kinases (such as PDK1) and causing its subsequent activation by phosphorylation (reviewed in reference 15).

Targets of activated Akt include the proapoptotic protein Bad, which is sequestered in its phosphorylated form by the 14-3-3 protein (thereby promoting cell survival) and by forkhead transcription factors that are retained in the cytoplasm when phosphorylated, thereby preventing transcription of forkhead target genes which include Fas ligand and insulin response sequences in the IGF-BP1 promoter (11, 31, 52). The transcription of genes such as IGF-I, IGF-II, IGF-II receptor, IGF-BPs, PI3K, PDK1, Akt, SGK, 14-3-3 protein, forkhead transcription factors (HFH-1, HFH-BF3, HZF-3), and Bcl-2 family genes including Bad were found to change upon infection by PRV and/or HSV-1 (Table 5). At 12 hpi, the expression of some of these genes changed in a proapoptotic direction, while others did so to promote cell survival, suggesting the existence of a fine balance between the two pathways in infected cells at late time points.

IFN- and IL-1-related genes were regulated primarily by HSV-1 infection and not by PRV infection. Our failure to see more IFN- and IL-1-related genes affected by PRV infection is both surprising and intriguing. Future studies might shed light on the significance of these differences in the IFN- and IL-1-stimulated responses elicited by the two virus infections and clarify whether they might underlie differences in pathogenicity in rodent infections by these viruses.

Many oxidative stress-stimulated genes were regulated by PRV and HSV-1 infections. Perhaps this result stems from a cytoprotective response effected by either the host or possibly the virus to counter hypoxic stress induced by infection. The antioxidant glutathione has recently been shown to inhibit the growth of influenza virus (13), a finding that underscores the importance of regulating oxidation during a productive viral infection.

We have assumed that the coordinate regulation of transcripts in a pathway is an indication that the proteins are actually produced and are functional. This assumption must be tempered with the knowledge that both HSV and PRV express proteins that affect mRNA stability, transport, and translation. We have verified that at least two transcripts induced late after infection by PRV and HSV infection from the *cox-1* and *cox-2* genes are functional, yielding increased amounts of Cox-1 and -2 proteins that are easily detected by Western blot analysis. Moreover, inhibitors of Cox-1 and -2 enzyme activity significantly reduce the yield of both viruses in several cell types (our unpublished results).

The virion host shutoff (Vhs) protein, which enters the cells as part of the virion tegument, stimulates nonspecific degradation of cellular mRNAs early in PRV and HSV-1 infection. In view of this activity, our observations and those of Taddeo et al. (51) that relatively few genes displayed reduced expression early after infection may seem contradictory. However, the modest early host shutoff observed may be linked to the growth-arrested state of the primary fibroblasts used in both studies. It is conceivable that compared to cycling cells, which have been used to demonstrate Vhs activity, growth-arrested cells might be less transcriptionally active and therefore display a less dramatic Vhs-induced decrease in mRNA levels—at least early in infection. At late times postinfection with either PRV or HSV-1, the expression of a large number of genes decreased more than threefold from that of the mock-infected group. As suggested by Taddeo et al., this finding could be the result of delayed Vhs activity or RNA decay in the absence of de novo transcription (51). Another reason might be that the newly synthesized Vhs protein is more abundant than that found immediately after infection. At late times postinfection, we also observed the increased expression of a large number of

TABLE 6. Genes that increase more than threefold by HSV-1(F) infection of rat and human fibroblasts

Gene		REF cells		HFF[b] cells	
GenBank no.	Description	MFI[a]	Time (hpi)	MFI	Time (hpi)
X13722	LDL receptor	3.75	0	7.8	3
X03369	Beta-tubulin	p 4.71	5	4.1	12
Z27118	Hsp70	26.34	12	6.3	12
AI176710	Nuclear receptor 4A3	p 14.42	12	10.1	1
X17012	IGF-II	p 13.08	12	6	12
M34253	IRF-1	10.68	12	7.2	7
L01624	SGK	p 8.21	12	4	7
AI230256	Id2	p 8.13	12	33.2	7
AF065431	BimL	7.56	12	14.2	7
AI014163	IFN-related development regulator 1	6.64	12	16	12
X06769	c-fos	p 6.4	12	35.3	1
AI639441	eIF-2	5.8	12	4.8	12
X07320	Phosphorylase kinase-gamma	p 5.59	12	11.7	12
AI008131	Amd1a	5.13	12	5.6	12
AA800613	Zfp36/Tis11	p 4.69	12	9.4	7
M60921	Btg2	p 4.63	12	8	7
L23148	Id1	4.56	12	26.2	7
J04197	Fruct-2,6-bisphosphatase	p 4.48	12	42.3	3
U64705	eIF4A	4.44	12	3.3	7
U17013	Oct1	4.3	12	4.2	7
AA955859	Splicing factor, Arg/Ser-rich 10	4.18	12	5.4	12
AF005099	Neuronal pentraxin receptor	3.85	12	4.6	7
AF000942	Id3	3.78	12	3.8	7
AA848218	DNA topoisomerase 1	3.58	12	7.1	7
AA875165	EST, gamma-tubulin complex	3.54	12	4.1	7
X96437	PRG1/IEX-1	p 3.5	12	4.6	7
AA850734	VEGF[c]	3.03	12	7.7	7
U75397	Krox24 (NGFI-A or Egr1)	p 3.03	12	3.3	12
D26500	Dynein-like protein 9	3.02	12	75	1
D49708	RNA-BP (transformer 2 like)	3.01	12	8.4	12

[a] MFI, maximum fold increase; "p" in front of the MFI indicates that the gene was also up-regulated in PRV-infected REF cells.
[b] HFF, human foreskin fibroblast.
[c] VEGF, vascular endothelial growth factor.

genes for both PRV and HSV-1. These transcripts increased in the face of Vhs activity and must be either induced to high levels by cellular pathways or are resistant to Vhs action and subsequent degradation.

Our analysis provides an opportunity to compare cellular gene expression induced by HSV-1 infection in rat and human fibroblasts. As mentioned above, changes in cellular gene expression induced by HSV-1 infection in growth-arrested human foreskin fibroblast cells was reported recently (51). We compared this data set with ours to extract a set of 29 genes whose expression is increased more than threefold compared to that of the mock-infected group upon HSV-1 infection of rat and human fibroblasts (Table 6). A subset of these genes also exhibited increased expression (more than threefold) after PRV infection of REF cells and are indicated as such in the same table. Expression of these genes might constitute part of an alphaherpesvirus signature. The same comparative analysis also allowed us to prepare lists of genes that are induced by HSV-1 infection in a cell-type-specific manner (supplemental information is available at http://www.molbio.princeton.edu/labs/enquist/SupplInfo.html). The genes induced only in the human cells by HSV-1 infection may be part of the response that contains spread in the natural host so that infection is mild and rarely lethal. On the other hand, genes that are induced only in the rat cells may reflect the response of a nonnatural host to infection by a human virus and provide insight into the process of the acute and often lethal infection that HSV-1 causes in rodents.

In summary, this work represents our first comparative analysis of the global cellular transcriptional changes induced in a common cell type by two alphaherpesviruses. We believe that these analyses may lay the foundation for larger comparative studies aimed at assessing the molecular signatures of herpesvirus infection in different cell types and hosts. PRV and HSV-1 are distantly related alphaherpesviruses that engage and modulate a multitude of cellular processes to achieve a productive infection. The primary question we asked was whether the two viruses engage the same or different pathways in a common cell type. Obviously, the outcome of infection in vivo depends on the response of infected cells, the singular event that triggers a cascade leading to spread or containment of the infection. A striking finding of our study was the large increase in cellular transcription changes late in infection. While we often consider the early modulation of host defenses essential to establishment of a productive infection, the cellular events that happen late in infection, when early cellular defenses have been breached, are not well understood. The late changes observed in stress response and heat shock genes might be involved in sending out general alarm signals to the immune system, reporting the imminent danger induced by virus infection. However, the late response is more than a general stress response because more than one-third of these

late changes are virus specific. It is now of some interest to determine if these cellular transcripts are functional, if different cell types have similar or different responses, and if the resulting cellular proteins influence the infection in a given animal.

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