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Gene Expression during Reactivation of Herpes Simplex Virus Type 1 from Latency in the Peripheral Nervous System Is Different from That during Lytic Infection of Tissue Cultures

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Herpes simplex virus (HSV) replicates in peripheral tissues and forms latent infections in neurons of the peripheral nervous system. It can be reactivated from latency by various stimuli to cause recurrent disease. During lytic infection in tissue culture cells, there is a well-described temporal pattern of (i) immediate-early, (ii) early, and (iii) late gene expression. However, latency is characterized by little if any expression of genes of the lytic cycle of infection. During reactivation, the pattern of gene expression is presumed to be similar to that during the lytic cycle in tissue culture, though recent work of W. P. Halford et al. (J. Virol. 70:5051–5060, 1996) and P. F. Nichol et al. (J. Virol. 70:5476–5486, 1996) suggests that it is modified in neuronal cell cultures. We have used the mouse trigeminal ganglion explant model and reverse transcription-PCR to determine the pattern of viral and cellular gene expression during reactivation. Surprisingly, the pattern of viral gene expression during lytic infection of cell cultures is not seen during reactivation. During reactivation, early viral transcripts were detected before immediate-early transcripts. The possibility that a cellular factor upregulates early genes during the initial reactivation stimulus is discussed.

Herpes simplex virus (HSV) establishes latent infections within the neuronal cell bodies of sensory ganglia that innervate the primary site of infection (10, 11). Latency is distinguished from acute infection by the lack of detectable infectious virus, or viral polypeptides. Although complete genomes are detected in the ganglia (37), viral transcription is limited to the latency-associated transcripts (LATs) (reviewed in references 18 and 60), whose function in establishing or maintaining latency is not well understood. HSV can undergo periodic reactivation in humans and in laboratory animals to produce recurrent disease, typified by mucocutaneous lesions at the peripheral site innervated by the infected ganglion (reviewed in reference 51). The mechanisms of transcriptional activation which govern the reactivation of virus from latency remain unclear.

A sequential regulatory cascade of HSV gene expression occurs during productive infection in vitro (22) and is assumed to occur at the primary sites of viral infection in vivo. Immediate-early (IE or α) viral gene products (infected cell polypeptides 0 [ICP0], ICP4, ICP27, ICP47, and ICP22) are expressed in the absence of prior viral protein synthesis (22, 23, 64). Expression of these genes is required for the expression of early (or β) and late (or γ) genes (9, 23). Efficient expression of late gene products in tissue culture cells requires viral DNA replication (21, 46).

Recent studies in primary cultures of neurons (20, 41) and in vivo (27, 28) suggest a different pattern of HSV gene expression. Expression of viral IE and early genes was at a basal level in neuronal cells treated with pharmacologic inhibitors of DNA replication or infected with replication-defective HSV type 1 (HSV-1) mutants (27, 41). These studies propose that efficient expression of all kinetic classes of HSV genes in neuronal cells requires DNA replication (27, 41). From these models, initiation of viral DNA replication is proposed to determine whether HSV infection follows the lytic or latent pathway (41).

Previous studies by us and others have demonstrated that viral genes are expressed within the first 24 h following explantation of latently infected trigeminal ganglia (TG), as detected by in situ hybridization, protein analysis, and reverse transcription-PCR (RT-PCR) (4, 14, 28, 64, 66). However, which viral gene products appear first at the onset of reactivation, and the subsequent pattern of viral gene expression, are unknown.

During acute infection, IE viral gene expression is dependent on the interaction of cellular transcription factor Oct-1 with binding sites in the IE promoters (42). Oct-1 and a second ancillary cellular protein (HCF) interact with the viral transactivator VP16 to form a complex that binds to octamer motif-TAATGARAT sequences upstream of all IE gene promoter transcriptional initiation sites (30, 31, 45, 60). This complex is essential for activation of IE transcription during acute infection (1). However, VP16 does not appear to be required for reactivation from TG explants (58). These observations suggest either that the VP16-activating function is not required for the initial steps of reactivation or that a cellular gene product substitutes for its function during reactivation.

We have previously reported that cellular transcription factors Oct-1, c-Jun, and c-Fos are induced in neuronal and nonneuronal cells after explantation of latently infected TG (63). Others have shown that cellular gene products complement a defect in the viral transactivator ICP0 in vitro (6, 67). Taken together, the data suggest that altered expression of cellular factors in sensory neurons leads to induction of HSV gene expression, resulting in reactivation. However, the role of viral IE genes in the reactivation process and subsequent viral gene expression is unclear.

In this study, we have used RT-PCR to determine the tem-

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TABLE 1. Primer pairs used in this study

Name	Gene	Sequence	PCR product (bp)	Reference
LAT		GACAGCAAAAATCCCCTGAG		29
		ACGAGGGAAAACAATAAGGG	195	
ICP27A[a]	UL54	TGCGGCCCTTTCTCCAGT		
		TGCGTGTCTAGGATTTCG	260	
ICP27B	UL54	TTTCTCCAGTGCTACCTGAAGG		14
		TCAACTCGCAGACACGACTCG	283	
ICP0	IR-S1	TTCGGTCTCCGCCTGAGAGT	DNA, 922	32
		GACCCTCCAGCCGCATACGA	cDNA, 157	
ICP22[a]	US1	AAGTAAACATCTGGGTCG		
		ACCGTGACCGACAGTCCC	159	
ICP47[a]	US12	ATCAATAAAAGGGGGCGT		
		ACTGTCGGTCACGGTCCC	159	
ICP4		GGCGGGAAGTTGTGGACTGG		14
		CAGGTTGTTGCCGTTTATTGCG	138	
ICP6-RR	UL39	GACAGCCATATCCTGAGC		14
		ACTCACAGATCGTTGACGACCG	221	
VP5	UL19	TGAACCCCAGCCCCAGAAACC		2a
		CGAGTAAACCATGTTAAGGACC	149	
TK	UL23	ATGGCTTCGTACCCCTGCCAT		55
		GGTATCGCGCGGCCGGGTA	531	
UL9-OBP	UL9	GGTCGTTCATAATGTAATTGG		12a
		CCGGCATAAGAGCTCGCCGTGGCG	559	
VP16	UL48	TGGGCAGCGTTGATAGGAAT		55
		GTTTGGGGGTTTTCTCTTCC	437	
gCA[a]	UL44	GAAACTGCCTCCACCGGGC		
		GGCGTCACCTCGCCGATAATC	603	
β-Actin		ATAGCACAGCTTCCCTTTGAT		12a
		AACATGCATTGTTACCAACT	452	
Cyclophilin		ATTCGAGTTGTCCACAGTCAGCAATGG		55
		ATGGTCAACCCCACCGTGTTCTTCGAC	469	
Oct-1[a]		AGACTCCTAAGCACTTCCCAGC		
		CACCTTGATTGCTGAACAGCT	306	
c-Jun[a]		TGTCGCAACCAGTCAAGTTCTC		
		TGCAAGCCCTGAAGGAAGAGC	411	
c-Fos		GAATAAGATGGCTGCAGCCAAGTGC		65
		AAGGAAGACGTGTAAGCAGTGCAGC	698	
c-Myc		CAAGAGGCGAACACACACAACGTCT		26
		AACTGTTCTCGTTTCCGCAA	218	

[a] Primer pairs were designed based on available sequences.

poral order of viral gene expression during explant reactivation of HSV-1. Surprisingly, only early viral gene products were detected at the earliest times after explantation.

MATERIALS AND METHODS

Virus stocks. To produce virus stocks, subconfluent monolayers of baby hamster kidney 21 clone 13 (BHK) cells were infected with HSV 17^+ (5) or HSV-1 F (15). The titers of viral stocks used were 2.3×10^8 PFU/ml for HSV 17^+ and 1.5×10^9 PFU/ml for HSV-1 F.

Infection of mice and explant reactivation. Four- to six-week-old female BALB/c BYJ mice were obtained from the Jackson Laboratory. Mice were anesthetized with intraperitoneal injection of ketamine (87 mg/kg)-xylazine (13 mg/kg) and then inoculated after corneal scarification with 10^4 to 10^5 PFU of HSV-1 17^+ or HSV-1 F per eye. At a minimum of 28 days postinfection, mice were sacrificed by cervical dislocation, and TG were obtained. Groups of six explanted TG were incubated in Dulbecco modified Eagle medium supplemented with 5% fetal bovine serum at 37°C for 0, 1, 2, 4, 8, 24, 48, 72, or 96 h postexplantation (p.e.). TG were incubated in the absence of serum in one experiment.

In vitro infection of TG. Groups of six uninfected TG explants were incubated in 1 ml of serum-free medium containing 10^6, 10^4, 10^3, or 10^1 PFU of HSV 17^+ per ml for 30 min at 37°C. After a brief wash in ice-cold phosphate-buffered saline, ganglia were incubated for 0, 1, 2, and 4 h in culture medium at 37°C and then snap-frozen in liquid nitrogen or processed for immunohistochemistry.

Extraction of RNA and reverse transcription. Ganglia used for RNA preparation were snap-frozen in liquid nitrogen. RNA was isolated from TG and brainstems by using the TRIzol reagent as described by manufacturer (Gibco BRL) followed by extensive digestion with RNase-free DNase I (Boehringer Mannheim Biochemicals) and ethanol precipitation. cDNA was generated from 2 mg of total RNA by using Superscript preamplification kit priming with oligo(dT) and random hexamers (Gibco BRL).

PCR amplification of cDNA or DNA. Reactions were performed in 25-μl volumes containing 4% cDNA, or 10 ng of DNA, 200 mM each deoxynucleoside triphosphate (Pharmacia), 1 μM each primer, and 2.5 U of *Taq* polymerase with PCR buffer A (Fisher). Primer pairs used are described in Table 1. Primers for ICP27A, ICP47, ICP22, β-actin, Oct-1, and c-Jun, were designed on the basis of available DNA sequence data (3, 34, 35, 52, 59). Cycling reactions were performed with a Perkin-Elmer (Norwalk, Conn.) thermal cycler. After one cycle of 4 min of denaturation at 94°C, cycles were as follows: (i) 1 min of denaturation at 94°C, (ii) annealing at 60°C for 1 min, and (iii) extension for 2 min at 72°C. The final cycle was terminated with a 7-min extension at 72°C. Amplification was carried out for 35 to 40 cycles. Negative controls of cDNA prepared from uninfected animal TG explants and reactions done without reverse transcription were included in each set of experiments.

PCR standards. HSV 17^+ DNA was purified from infected Vero cells as described previously (57). Viral DNA (0.6×10^6 to 6×10^6 copies/ml) was serially diluted in mouse DNA prepared from brains by using DNAzol reagent (Gibco BRL). A total of 10 ng (1 μl) was subjected to PCR with each primer set to compare primer sensitivity. To standardize the reverse transcription reaction, 1 μl of in vitro-transcribed β-globin RNA was added to each sample followed by PCR with specific primers provided with the Superscript PCR preamplification kit (Gibco BRL). Experiments were repeated twice.

Immunohistochemical procedures. Ganglia used for immunohistochemistry were immersed in 70% ethanol–150 mM NaCl for 24 h and then embedded in paraffin wax. Serial 6-μm sections were cut and processed as described elsewhere (49). Rabbit polyclonal antiserum to HSV-1 (Dako Corporation, Carpinteria, Calif.) was used for detection of replicating virus as described previously (2, 25). Mouse monoclonal antiserum 58S (54) was used to detect ICP4. Rabbit polyclonal anti-mouse Oct-1 (Santa Cruz Biochemicals, Santa Cruz, Calif.) was used to detect Oct-1. Antigen-expressing cells were detected by an indirect avidin-

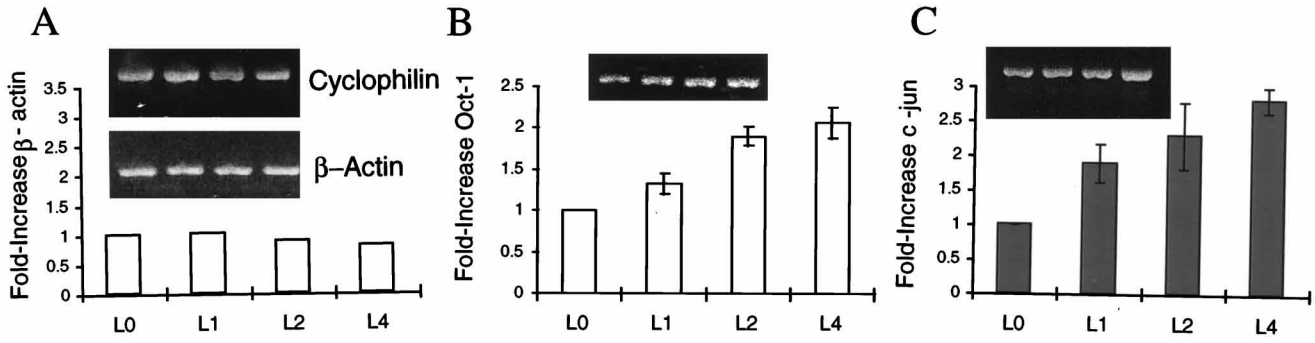

FIG. 1. RT-PCR detection of Oct-1 and c-*jun* transcripts in murine TG following explantation. RT-PCR was used to detect Oct-1, c-*jun*, β-actin, and cyclophilin mRNAs in duplicate samples of RNA from latently-infected TG at 0, 1, 2, and 4 h p.e. (L0 to L4). Products were separated by agarose gel electrophoresis, followed by Fluorimager scanning and analysis using Imagequant software. The relative amount of cDNA is expressed in arbitrary units representing the ratio between the intensity of the PCR product band (β-actin, Oct-1, or c-*jun*) and the intensity of cyclophilin. The ratio at the zero time point is designated 1. (A) β-Actin in latent explants; (B) Oct-1 in latent explants; (C) c-*jun* in latent explants.

biotin immunoperoxidase method (Vectastain ABC kit; Vector Laboratories, Burlingame, Calif.) with 3,3′-diaminobenzidine as the chromagen (62).

Detection of PCR products. Aliquots of 40% of amplification products were fractionated on 2.5% NuSieve agarose (FMC). Gels were stained with ethidium bromide (Sigma), and the amounts of products were quantitated by fluorimetry. The relative amount of PCR product was determined in arbitrary numbers as the ratio between the PCR product band intensity and that of a cellular housekeeping gene, encoding cyclophilin or β-actin (14). Statistical analysis was performed with Excel (Microsoft, Redmond, Wash.). To confirm the specificity of PCRs, products were separated by agarose electrophoresis, blotted onto nylon membranes (Boehringer Mannheim Biochemicals), and probed with biotinylated oligonucleotides specific for VP5, ICP27 (14), or LAT (29), using a Southern Lights kit (Tropix).

RESULTS

Mice were infected with HSV-1 following corneal scarification. Previous reports have shown that by 10 days postinfection, all evidence of the acute stage of viral gene expression in ganglia is gone (51), leaving only latent viral genomes. TG were surgically removed from mice 28 days postinfection and incubated in culture medium at 37°C as described in Materials and Methods. Using the highly sensitive technique of RT-PCR, we examined the temporal onset of viral gene expression and compared the pattern of appearance to that of cellular IE genes.

Cellular IE gene transcription can be detected by 2 h p.e. We previously reported that certain cellular IE genes such as Oct-1, c-*jun*, and c-*fos* are induced in neuronal cells between 10 to 48 h p.e., as detected by in situ hybridization (63). In this study, we used sensitive RT-PCR to determine the temporal onset of induction of cellular IE genes. RNA was prepared from TG explants at various times p.e. followed by RT-PCR for cellular genes as described in Materials and Methods. PCR products were separated by agarose gel electrophoresis, and the relative amounts of PCR products were evaluated by Fluorimager (Molecular Dynamics, Inc.) analysis (Fig. 1). There was no difference between the relative amounts of expression of cellular housekeeping β-actin and cyclophilin genes (Fig. 1A). However, a reproducible net increase in the relative amount of Oct-1 cDNA was detected within 2 h postexplantation in both infected and uninfected animals (Fig. 1B; data for uninfected animals not shown). This induction occurred earlier than previously observed (63) and, importantly, prior to the onset of viral gene expression (see below).

We next examined whether the moderate induction of RNA levels represents an increase in Oct-1 protein expression. Sections of TG from various times p.e. were probed by immunohistochemistry with an Oct-1-specific antibody. Oct-1 was markedly induced specifically in all neuronal cells (Fig. 2). Since recent in situ PCR studies have shown that the majority of TG neurons which innervate the cornea harbor HSV-1 DNA (36, 47, 48), Oct-1 was induced in latently infected neurons. Moreover, neurons represent approximately 10% of the TG cell population, which may explain the fact that only a twofold induction was detected by RT-PCR (Fig. 1B).

The induction of c-*jun* was detected previously by in situ hybridization at 10 h p.e. (63). Using RT-PCR, we detected an increase in c-*jun* transcription at 1 h p.e. (Fig. 1C). Similar results were obtained for uninfected explants (not shown). Moreover, we found that c-*fos* and c-*myc* were induced within the first 4 h p.e., whereas the relative levels of cDNAs for the cellular housekeeping β-actin and cyclophilin genes remained unchanged (Fig. 3). Clearly, Oct-1, c-*jun*, c-*fos*, and c-*myc* are induced in the absence of latent virus and thus are not a response to viral gene expression. These experiments were repeated with RNA prepared from TG incubated in serum-free medium. Induction occurred under both conditions (results not shown), indicating that cellular IE genes were induced by the stress of explantation, not by serum proteins in the explantation medium (52).

Transcription of viral genes during reactivation. To determine the temporal order of viral gene expression during reactivation, we used RT-PCR. At various times ranging from 0 to 96 h p.e., RNA was prepared from six pooled TG and analyzed by RT-PCR.

As expected, immediately after explantation (28, 29), only LAT cDNA was detectable (Fig. 4; Table 2). Within the first 4 h p.e., thymidine kinase (TK), ICP6 (large subunit of ribonucleotide reductase), and VP5 cDNA produced faint but clearly visible PCR products on ethidium bromide-stained agarose gels (data for ICP6 shown in Fig. 4 and Table 2). Qualitative comparison indicated an increase in the amount of these transcripts relative to the cellular housekeeping cyclophilin and β-actin genes (not shown). At 24 and 48 h p.e., late (γ) VP16 and gC transcripts were detected. Surprisingly, IE (α) transcripts ICP0, ICP22, ICP47, and ICP27 were detected by RT-PCR only after 24 h p.e. (results for ICP27 shown in Fig. 4C). Two separate sets of primers for ICP27 were used to confirm these results. The ICP27B primer was used by others (4, 14) to detect ICP27 by 2 h p.e. in TG of 17^{+}-infected Swiss Webster mice and rabbits; however, we detected ICP27 cDNA only at 24 h p.e. or later (Fig. 4C). A band of ICP4 was detected in cDNA prepared from a single group of animals at 8 h p.e. (not shown). Using a more sensitive enzyme, AmpliTaq

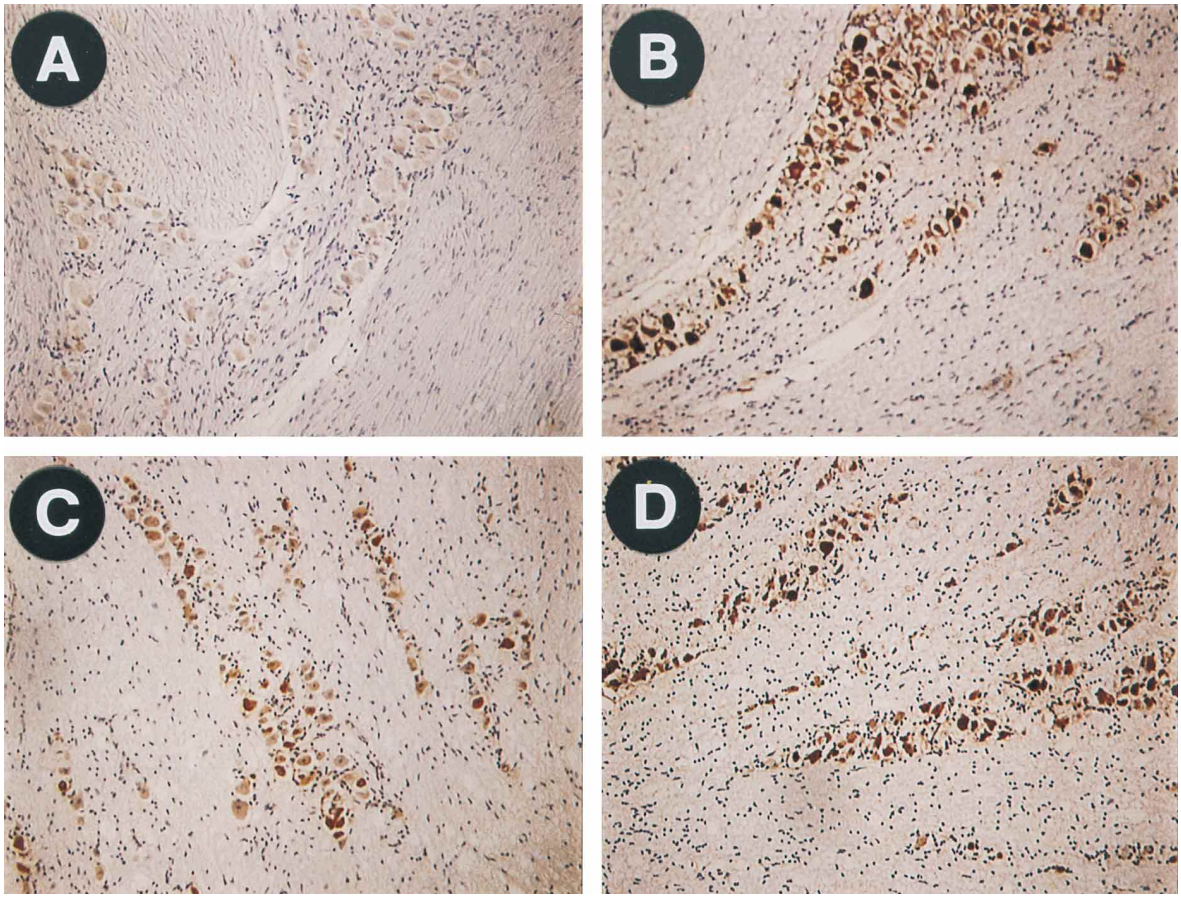

FIG. 2. Detection of Oct-1 protein in TG following explantation by immunostaining. Latently infected mice were sacrificed, and TG explants were incubated in culture medium for various times p.e. Paraffin-embedded serial sections were reacted with rabbit polyclonal antisera specific for Oct-1 and processed as described in Materials and Methods. (A) 0 h after explantation of latently infected TG; (B) 4 h; (C) 8 h; (D) 24 h. Magnification, ×20.

Gold (Perkin-Elmer), and the same primers and conditions, we detected viral transcripts ICP27 and ICP6 at 0 h p.e. (Fig. 5A). This result suggests that there may be a basal level of transcription and possible gene expression during HSV latency, in agreement with findings of Kramer and Coen (29). However, qualitative comparison indicated an increase in the relative amount of ICP6 cDNA at 2 and 4 h. In contrast, at the later time points, ICP27 levels remained at the basal levels (Fig. 5B). Overall, early gene induction was detected before that of IE genes in at least five groups of animals latently infected with two different strains of HSV-1.

Comparison of primer sensitivities. Since differences in primer sensitivity can cause variation in detection levels, we determined the ability of each primer set to amplify HSV DNA. Purified viral DNA was diluted in uninfected mouse DNA and subjected to PCR amplification. Primers specific for ICP47, ICP22, and TK detected only 10^4 copies of HSV DNA (Table 2). Primers for ICP27 detected similar amounts of HSV DNA (10 copies) as ICP6 primers (Fig. 6). Thus, our inability to detect an increase in the levels of the IE gene ICP27 at early times was not due to low primer sensitivity compared to that of the early gene primer sets.

Early viral proteins are detected by 4 to 8 h p.e. Since early viral RNA was detected by 4 h p.e., we wished to determine whether protein products were present. Serial sections of TG from 0 to 24 h p.e. were probed for TK, ICP4, and ICP0 polypeptides by using immunohistochemistry. TK was detected by 4 h p.e. in approximately 1% of neuronal cells and at increased levels at 8 to 24 h (Fig. 7). ICP4 was detected in a few neurons at 8 h p.e., whereas ICP0 was detected only at 24 h p.e. (not shown). We previously have reported that in the presence of inhibitors of DNA synthesis, viral gene products of approximately 43 kDa, the size of TK, can be detected in reactivating TG by immunoprecipitation with polyclonal HSV-1-specific antisera at 6 to 12 h p.e. (66). Viral proteins corresponding to 110 and 175 kDa, the sizes of ICP4 and ICP0, were detected only at 12 to 24 h p.e. (66). These results are consistent with our RT-PCR data, suggesting that IE proteins are not the first viral genes activated during reactivation.

In vitro infection of explanted ganglia. To determine whether IE transcripts can be detected prior to early genes during acute infection of the TG, intact TG explants were infected with 10^6 PFU of HSV-1 strain 17^+ per ml in 1 ml of serum-free medium for 30 min at 37°C. Under these conditions, the majority of infected cells were neuronal rather than astrocytes, as determined by immunohistochemistry for HSV antigens (not shown). All classes of viral transcripts were detected by RT-PCR at 90 min postinfection (Table 3). Thus, IE transcripts were detected simultaneously with early genes during acute infection. It is possible that transcription from both IE and early promoters was induced by viral transactivators introduced by the high number of input virions. To address this possibility, the experiments were repeated with 10^4, 10^3, and 10 PFU/ml. Expression of IE (ICP4) and early (ICP6) viral genes

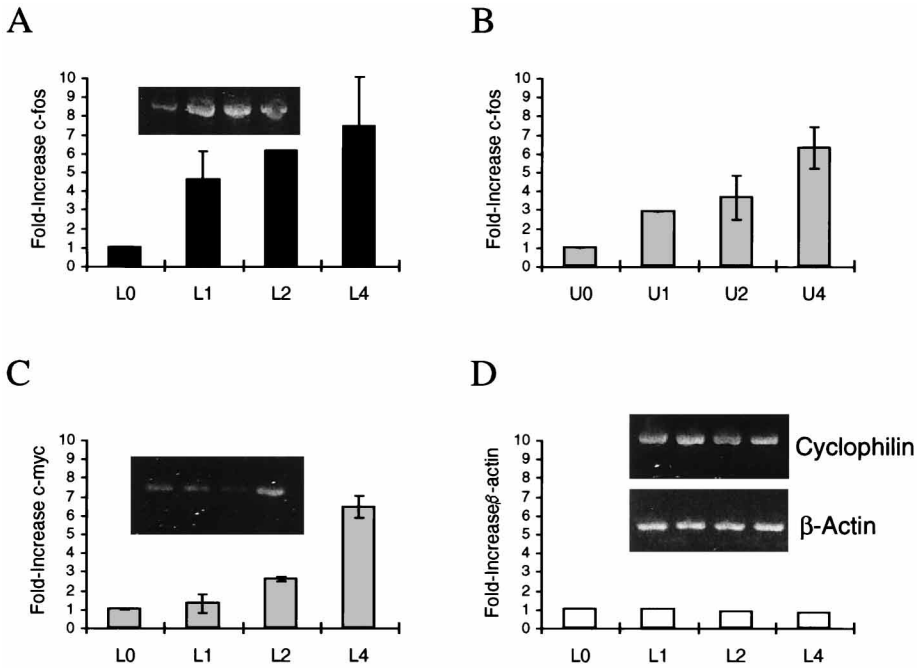

FIG. 3. RT-PCR detection of c-*fos*, c-*myc*, β-actin, and cyclophilin transcripts in murine TG following explantation. RNA from latently infected (A, C, and D) or uninfected (B) TG explants was prepared and analyzed by RT-PCR for c-*fos* (A and B) c-*myc* (C), and β-actin (D) as described in Materials and Methods. Products were visualized by ethidium bromide staining as shown in the insets. The graphs represent the ratio between the PCR product bands (c-*fos*, c-*myc*, and β-actin) and the cyclophilin band. The ratio at the time of explantation was designated 1. Experiments were done in duplicate. L1 to L4, latent, 1 to 4 h postexplant; U1 to U4, uninfected, 1 to 4 h postexplant.

was detected at the same time points (1 to 2 h postinfection [results not shown]). These results indicate that both IE and early gene promoters are activated during the onset of acute infection of TG explants, similar to findings for primary cultures of neuronal cells (41).

DISCUSSION

HSV expresses its genes in a regulated ordered cascade during lytic infection. During latency, genes of the viral lytic cycle are not expressed or show only basal levels of expression (12, 29). Upon reactivation of the latent genome, viral gene expression is once again activated, leading to the production of infectious virus. The nature of the cellular stimuli which reactivate the latent viral genome is the subject of much speculation and several hypotheses. It is thought that a cellular transcription factor, or factors, may be upregulated by the reactivation stimulus, causing activation of viral and cellular genes. A candidate for this factor would be an Oct-1-like activator which could bind to any of the octamer-like motifs in viral IE gene promoters, resulting in induction of these genes and entry into the lytic infection cycle. In a variation of this hypothesis, a cellular factor with properties similar to those of VP16 may be involved in octamer factor activation at the viral IE gene promoters.

Other cellular factors may induce viral IE genes, leading to initiation of the viral lytic infection cycle. However, it is possible that the reactivation stimulus does not specifically act on viral IE genes but rather activates multiple kinetic classes of viral genes. There are consensus binding sites for transcription factors, such as Sp1, throughout the viral genome and in most viral IE and early promoters (reviewed in reference 51). Furthermore, it has been proposed that viral transcription is activated through a mechanism involving upregulation of promot-

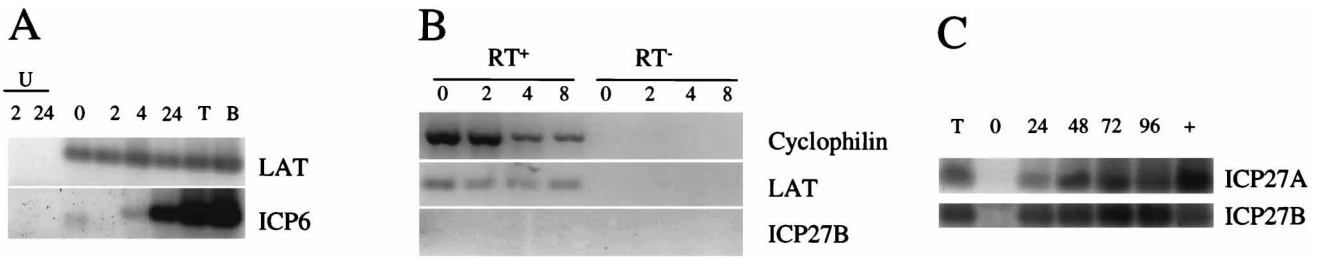

FIG. 4. RT-PCR detection of viral gene expression in latently infected explanted TG. Latently infected BALB/c mice were sacrificed, and TG were removed aseptically. Following incubation in culture medium for 0 to 96 h, cDNA was prepared as described in Materials and Methods followed by 35 cycles of PCR amplification. Products were analyzed by agarose gel electrophoresis and Fluoroimager scanning. Shown are amplification products from primer sets specific for LAT and ICP6 primers (A), ICP27B (B), and ICP27A and ICP27B (C). See Table 1 for primer sequences. cDNA prepared from uninfected TG (U) and RT^- (no reverse transcription) samples were used as negative controls in each experiment. cDNAs prepared from mice TG (lane T) and brainstems (lane B) at day 3 postinfection and 5 ng of purified HSV-1 DNA (lane +) were used as positive controls.

TABLE 2. RT-PCR on RNA isolated from reactivating mouse TG explants

Time (h) p.e.	Reaction[a]										
	α-0	α-4	α-27	α-22	α-47	ICP6	VP5	TK	gC	VP16	LAT
0	−	−	−	−	−	−	−	−	−	−	++
2	−	−	−	−	−	−	−	−	ND	ND	++
4	−	−	−	−	ND	+	+	+	ND	ND	++
5[b]	−	ND	−	ND	ND	+	+	ND	−	−	++
8[b]	−	−/+	−	ND	ND	ND	++	ND	ND	−	++
24	+/−	++	++	−	−	++	++	++	−	+	++
48	+	++	++	ND	ND	++	++	ND	+	++	++
72	+	++	++	+	+	++	++	ND	++	ND	++
96	+	++	++	ND	ND	++	++	ND	++	++	++
Uninfected	−	−	−	−	−	−	−	−	−	−	−
Primer sensitivity[c]	ND	10^2	10^1	10^4	10^4	10^1	10^3	10^4	10^2	10^4	10^4

[a] −/+, positive in some experiments; +, weak positive band; ++, strong positive band; ND, not determined. All samples were positive for β-actin and cyclophilin.
[b] Results for strain 17^+ only.
[c] Defined as the minimum number of HSV DNA molecules detected by each primer set.

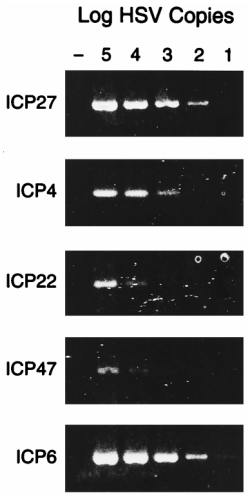

FIG. 6. PCR assay of primer sensitivity. Purified HSV-1 DNA was serially diluted in mouse DNA to a final DNA concentration of 10 ng/ml. DNA samples were subjected to PCR with the indicated primers, using the conditions described in Materials and Methods. Reactions lacking template DNA were used as negative controls in each experiment. Products were analyzed by agarose gel electrophoresis and ethidium bromide staining. The amount of specific HSV DNA is indicated.

ers located at an origin of viral replication (51). In this study, we used RT-PCR to examine the order of expression of viral and cellular genes following a reactivation stimulus and showed that early viral transcripts are the first to be detected.

IE cell factors are induced by the stress of explantation. There are many model systems with which to study reactivation, including the HSV-1 rabbit eye spontaneous and iontophoresis-induced reactivation model, the HSV-2 guinea pig vaginal model, and the mouse explant model (reviewed in reference 51). These models are presumed to involve reactivation of the latent virus by similar mechanisms, although the strength of the inducing stimulus or the detailed pathway by which the viral activity factors are induced may be different. The mouse ocular model has the advantage that it does not involve spontaneous reactivation at any measurable level without physical or chemical induction and thus is useful for studies of gene expression during latency and following reactivating stimuli. Previous studies from our lab have shown that cellular genes such as c-*fos*, c-*jun*, and Oct-1 are upregulated in murine TG by the stress of explantation (63).

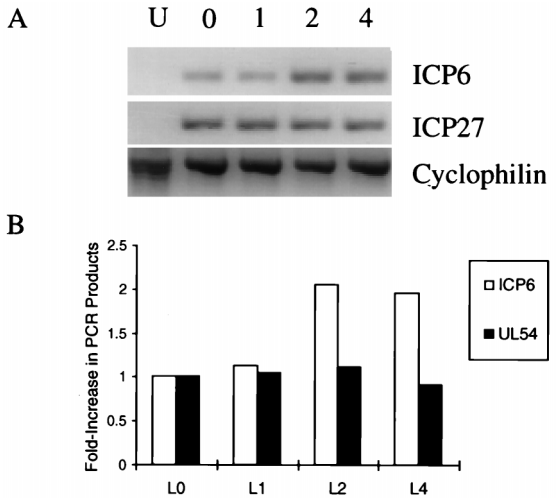

FIG. 5. Detection of basal viral transcription in latent and reactivating TG by sensitive RT-PCR. Latently infected or uninfected (lane U) BALB/c mice were sacrificed, and TG were removed aseptically. Following incubation in culture medium for 0 to 4 h (L0 to L4), cDNA was prepared as described in Materials and Methods. Samples were then subjected to 40 PCR cycles using the enzyme AmpliTaq Gold. Products were separated by agarose gel electrophoresis, followed by Fluorimager scanning (A) and analysis using Imagequant software (B). The relative amount of cDNA is expressed in arbitrary units representing the ratio between the intensity of the PCR product band and the intensity of cellular housekeeping cyclophilin gene. The ratio at the zero time point is designated 1.

In this study, using RT-PCR, we have shown that we can detect increases in the levels of Oct-1 transcripts relative to the cellular housekeeping cyclophilin and β-actin genes at early times p.e. (Fig. 1). Oct-1 was shown to rise by 1 h p.e. and to continue rising through 4 h p.e. This result agrees with and extends our previous findings from in situ hybridization studies using both latently infected and uninfected TG (63). Immunohistochemical staining using an antibody specific for Oct-1 confirmed an increase in Oct-1 protein and showed that the induction is primarily in neuronal cells (Fig. 2). Similarly, *jun*, *myc*, and *fos* transcription was increased following the explantation (Fig. 1 and 3). Thus, it can be concluded that the stress of explantation induces many cellular IE genes in both latently infected and uninfected TG, some of which may act upon the viral genome to cause reactivation. Importantly, these increases in cellular IE gene expression were detected prior to viral gene expression. Thus, these genes are candidates for activators of the viral genome during reactivation.

Viral factors induced during reactivation. Expression of viral early transcripts (TK, ICP6, and VP5) was detected within the first 4 h following explantation of latently infected murine TG. Surprisingly, no induction of IE transcription was detected until 8 to 24 h p.e. (Fig. 4; Table 2). These data suggest that the cellular stimulus causing reactivation does not act specifically on the IE class of viral genes but rather acts on a broader class of viral genes.

However, these data represent times at which gene expression is detected; they are not measurements of the times at which absolute on/off switches in transcription are occurring. Thus, it is possible that extremely low (basal) levels of tran-

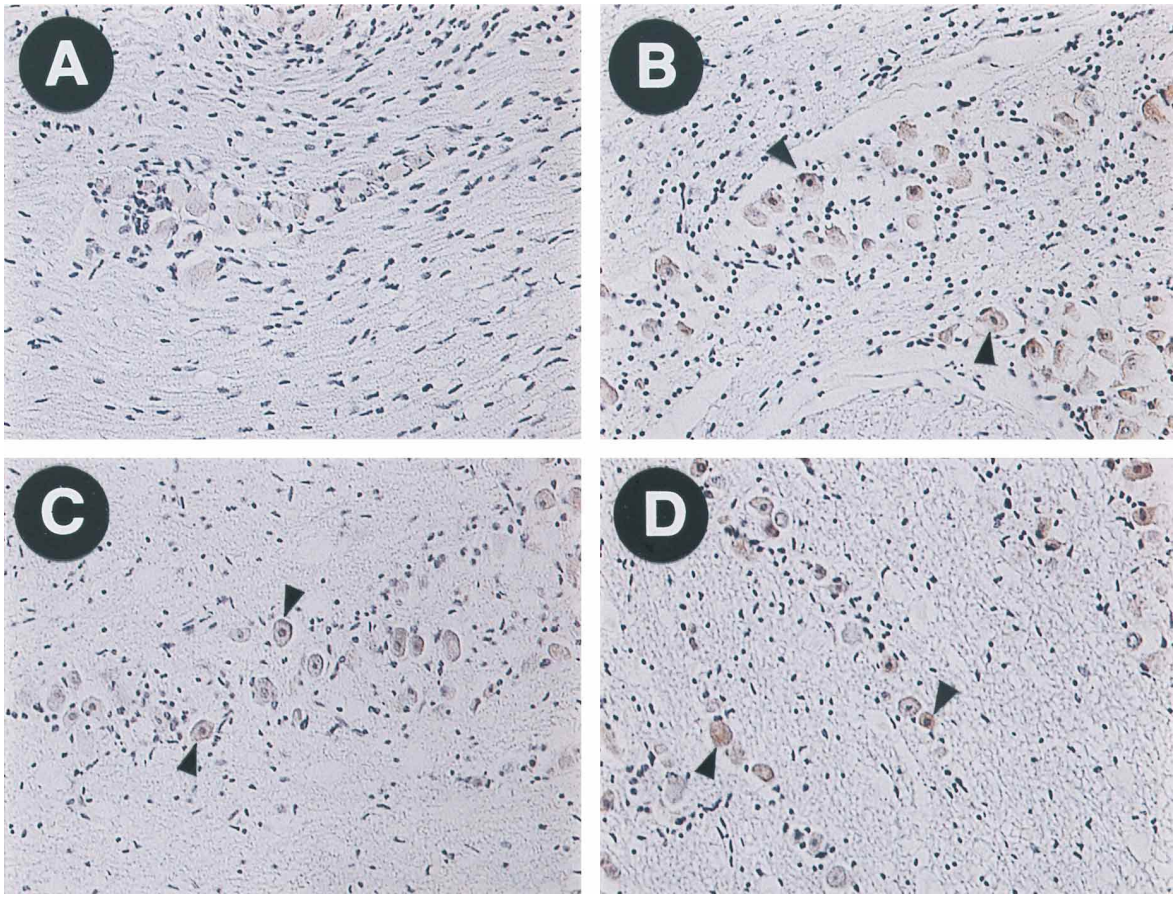

FIG. 7. Immunohistochemical detection of TK in latently infected TG following explantation. Latently infected mice were sacrificed, and TG explants were incubated in culture medium for varying times p.e. Paraffin-embedded serial sections were reacted with a rabbit polyclonal antiserum directed against TK and processed as described in Materials and Methods. (A) 0 h; (B) 4 h; (C) 8 h; (D) 24 h. Magnification, ×20.

scription occur from the viral IE genes which do not accumulate to detectable levels until 24 h p.e. Moreover, viral early genes may be transcribed more efficiently, allowing for measurement of detectable levels at early time points. Furthermore, it is possible that the level of detection varies with the relative sensitivity of the PCR primer selected. We have compensated for the latter variable by comparing the primer sensitivities (Fig. 6; Table 2).

Viral transcription following in vitro infection. Viral gene expression following infection of multiple cell types in culture is well characterized as a cascade of IE, early, and late genes (for a review, see reference 51). However, it has been suggested that the regulation of HSV gene expression in neuronal cells of the TG is different from the regulatory cascade in cell types such as Vero cells (27). Other studies have also found different patterns of viral gene expression in neuronal cell tissue culture experiments (20, 38). These data are consistent with our findings that induction of early genes was detected before that of IE genes during reactivation. Furthermore, they support the hypothesis that the factor which induces viral gene expression during reactivation acts on more than the five viral IE gene promoters. In fact, efficient IE gene expression did not occur until 24 to 48 h p.e., when DNA replication has been previously detected (14, 56). Recently, the importance of viral DNA replication on IE and early viral gene expression in neuronal cells has been suggested by Nichol et al. (41). A mutation in UL9, a DNA binding protein essential for viral DNA replication, was shown to affect the efficiency of transcription from the ICP6 promoter in primary cultures of rat neurons. Furthermore, treatment with acyclovir inhibited efficient ICP0 expression (41).

TABLE 3. RT-PCR for viral transcripts in mouse TG infected in vitro[a]

Time (min) postinfection	Reaction[b]											
	α-0	α-4	α-22	α-47	α-27	ICP6	VP5	UL9	TK	VP16	gC	LAT
30	−	−	−	−	−	−	−	−	−	−	−	−
90	+	+	+	−	+	+/−	−	+	+	−	+	+
150	+	+	+	−	+	+	+	+	+	+	+	+
210	+	+	+	+	+	+	+	+	+	+	+	+

[a] TG explants were infected with 10^6 PFU of HSV-1 strain 17^+ per ml. At various times postinfection, TG were snap-frozen, and RNA was prepared and subjected to RT-PCR.

[b] See Table 2, footnote *a*.

During acute infection, the viral genome is not associated with nucleosomal histones (13, 50). In contrast, during latency, it is packaged in a compact chromatin structure (13, 40), which is likely to affect the ability of cellular transcription factors to access viral promoters. Efficient transcription may require a change in chromatin structure. This could result from either DNA replication or modification of nucleosomal histones. Furthermore, the altered cascade of gene expression from the

acute genome compared to that of the reactivating genome detected in this study may result from such structural differences in transcriptional templates.

Do cellular factors expressed during reactivation mimic the function of a viral gene? In mouse eye inoculation experiments, VP16 is essential for lytic infection in the peripheral nervous system (58). In the absence of a functional VP16 capable of transactivating the IE genes, no lytic infection is detected. However, a latent infection can be established. This latent infection reactivated normally, suggesting that a cellular factor induced by the reactivation stimuli replaced the function of VP16 (58). From results of tissue culture infection experiments, Cai and Schaffer (7) hypothesized that a cellular factor in neuronal cells may complement ICP0 or VP16 and initiate the viral gene expression which is required for reactivation. The major candidates for activation by these cellular factors are the viral transactivator VP16, a protein encoded by a late gene, and IE gene products (6). However, we found only early viral transcripts induced during the first 24 h p.e. (Table 2). This result suggests that the cellular factors which induce reactivation in the TG activate early, rather than IE or late, promoters. Moreover, transgenic mice expressing metallothionein-driven VP16 were not different from their nontransgenic siblings with respect to latency, suggesting that VP16 is not sufficient for reactivation (53).

ICP0 is a promiscuous viral transactivator that regulates all kinetic classes of HSV-1 genes as well as a number of cellular genes (7, 8, 16, 17, 19, 33, 39, 43, 44). Is there a cellular factor substituting for the function of ICP0 in reactivating ganglia? The fact that all classes of HSV-1 genes are expressed during the onset of acute infection of TG explants (Table 3) supports this hypothesis. Moreover, in tissue culture experiments, a cellular factor which stimulates viral gene expression was found to substitute for ICP0 in NB41A3 cells upon release from growth arrest (46). It is also known that ICP0 activates AP-1 (24), components of which (Fos and Jun) were activated upon explantation (Fig. 1 and 3). Therefore, it is possible that a cellular protein induced in TG by the stress of explantation activates both viral and AP-1 promoters.

In conclusion, by using sensitive RT-PCR techniques, early viral gene induction can be detected before IE gene expression during reactivation from latently infected TG. This finding does not support the existence of a reactivation stimulus in which a cellular factor replaces the function of VP16 specifically upregulating IE genes. Rather, it supports the existence of a cellular factor that upregulates a broader class of genes. Moreover, we have shown that cellular IE genes are induced within the first hour following explantation. It would be of interest to determine whether these factors are involved in the reactivation stimulus.

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REFERENCES

1. **Ace, C. I., T. A. McKee, J. M. Ryan, J. M. Cameron, and C. M. Preston.** 1989. Construction and characterization of a herpes simplex virus type 1 mutant unable to transinduce immediate-early gene expression. J. Virol. **63:**2260–2269.
2. **Adams, R. L., D. R. Springall, M. M. Levene, and T. E. Bushell.** 1984. The immunocytochemical detection of herpes simplex virus in cervical smears—a valuable technique for routine use. J. Pathol. **143(4):**241–247.

2a. **Albelda, S.** Personal communication.

3. **Alonso, S., A. Minty, Y. Bourlet, and M. E. Buckingham.** 1986. Comparison of three actin-coding sequences in the mouse: evolutionary relationships between the actin genes of warm-blooded vertebrates. J. Mol. Evol. **23:**11–22.
4. **Bloom, D. C., G. B. Devi-Rao, J. M. Hill, J. G. Stevens, and E. K. Wagner.** 1994. Molecular analysis of herpes simplex virus type 1 during epinephrine-induced reactivation of latently infected rabbits in vivo. J. Virol. **68:**1283–1292.
5. **Brown, S. M., D. A. Ritchie, and J. Subak-Sharpe.** 1973. Genetic studies with herpes simplex virus type 1. The isolation of temperature-sensitive mutants, their arrangement into complementation groups and recombination analysis leading to lineage groups. J. Gen. Virol. **18:**329–346.
6. **Cai, W., and P. A. Schaffer.** 1991. A cellular function can enhance gene expression and plating efficiency of a mutant defective in the gene for ICP0, a transactivating protein of herpes simplex virus type 1. J. Virol. **65:**4078–4090.
7. **Cai, W., and P. A. Schaffer.** 1989. Herpes simplex virus type 1 ICP0 plays a critical role in the de novo synthesis of infectious virus following transfection of viral DNA. J. Virol. **63:**4579–4589.
8. **Cai, W., and P. A. Schaffer.** 1992. Herpes simplex virus type 1 ICP0 regulates expression of immediate-early, early, and late genes in productively infected cells. J. Virol. **66:**2904–2915.
9. **Clements, G. B., R. J. Watson, and N. M. Wilkie.** 1977. Temporal regulation of herpes simplex virus type 1 transcription: localization of the transcripts on the viral genome. Cell **12:**275–285.
10. **Cook, M. L., and J. G. Stevens.** 1973. Pathogenesis of herpetic neuritis and ganglionitis in mice: evidence for intra-axonal transport of infection. Infect. Immun. **7:**272–288.
11. **Dabrowski, C. E., P. J. Carmillo, and P. A. Schaffer.** 1994. Cellular protein interactions with herpes simplex virus type 1 oriS. Mol. Cell. Biol. **14:**2545–2555.
12. **Deatly, A. M., J. G. Spivack, E. Lavi, D. R. O'Boyle, and N. W. Fraser.** 1988. Latent herpes simplex virus type 1 transcripts in peripheral and central nervous system tissue of mice map to similar regions of the viral genome. J. Virol. **62:**749–756.

12a. **DelVecchio, F.** Personal communication.

13. **Deshmane, S., and N. W. Fraser.** 1989. During latency, herpes simplex virus type 1 DNA is associated with nucleosomes in a chromatin structure. J. Virol. **63:**943–947.
14. **Devi-Rao, G. B., D. C. Bloom, J. G. Stevens, and E. K. Wagner.** 1994. Herpes simplex virus type I DNA replication and gene expression during explant-induced reactivation of latently infected murine sensory ganglia. J. Virol. **68:**1271–1282.
15. **Ejercito, P. M., E. D. Kieff, and B. Roizman.** 1968. Characterization of herpes simplex virus strains differing in their effects on social behaviour of infected cells. J. Gen. Virol. **2:**357–364.
16. **Everett, R. D.** 1987. A detailed mutational analysis of VMW110, a trans-acting transcriptional activator encoded by herpes simplex virus type 1. EMBO J. **6:**2069–2076.
17. **Everett, R. D.** 1986. The products of herpes simplex virus type 1 (HSV-1) immediate early genes 1, 2, and 3 can activate HSV-1 gene expression in trans. J. Gen. Virol. **67:**2507–2513.
18. **Fraser, N. W., T. Block, and J. G. Spivack.** 1992. The latency-associated transcripts of herpes simplex virus: RNA in search of function. Virology **191:**1–8.
19. **Gelman, I. H., and S. Silverstein.** 1985. Identification of immediate early genes from herpes simplex virus that transactivate the thymidine kinase gene. Proc. Natl. Acad. Sci. USA **82:**5265–5269.
20. **Halford, W. P., B. M. Gebhardt, and D. J. J. Carr.** 1996. Mechanisms of herpes simplex virus type 1 reactivation. J. Virol. **70:**5051–5060.
21. **Harris-Hamilton, E., and S. L. Bachenheimer.** 1985. Accumulation of herpes simplex virus type 1 RNAs of different kinetic classes in the cytoplasm of infected cells. J. Virol. **53:**144–151.
22. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpes macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14:**8–19.
23. **Honess, R. W., and B. Roizman.** 1975. Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral polypeptides. Proc. Natl. Acad. Sci. USA **72:**1276–1280.
24. **Jang, K.-L., B. Pulverer, J. R. Woodgett, and D. S. Latchman.** 1991. Activation of the cellular transcription factor AP-1 in herpes simplex virus infected cells is dependent on the viral immediate-early protein ICP0. Nucleic Acids Res. **19:**4879–4833.
25. **Kesari, S., B. P. Randazzo, T. Valyi-Nagy, Q. S. Huang, S. M. Brown, A. R. MacLean, V. M.-Y. Lee, J. Q. Trojanowski, and N. W. Fraser.** 1995. Therapy of experimental human brain tumors using a neuroattenuated herpes simplex virus mutant. Lab. Invest. **73:**636–648.
26. **Kita, M., K. Tanaka, K. Shinmura, Y. Tanaka, Y. Liu, and J. Imanishi.** 1994.

Expression des genes des cytokines et des genes associes a l'interferon chez l'embryon de la souris. C. R. Soc. Biol. **188:**593–600.
27. **Kosz-Vnenchak, M., J. Jacobson, D. M. Coen, and D. M. Knipe.** 1993. Evidence for a novel regulatory pathway for herpes simplex virus gene expression in trigeminal ganglion neurons. J. Virol. **67:**5383–5393.
28. **Kosz-Vnenchak, M., D. M. Coen, and D. M. Knipe.** 1990. Restricted expression of herpes simplex virus lytic genes during establishment of latent infection by thymidine kinase-negative mutant viruses. J. Virol. **64:**5396–5402.
29. **Kramer, M. F., and D. M. Coen.** 1995. Quantification of transcripts from the ICP4 and thymidine kinase genes in mouse ganglia latently infected with herpes simplex virus. J. Virol. **69:**1389–1399.
30. **Kristie, T. M., J. H. Lebowitz, and P. A. Sharp.** 1989. The octamer-binding proteins form multi-protein DNA complexes with the HSV alpha TIF regulatory protein. EMBO J. **8:**4229–4238.
31. **LaMarco, K., and S. McKnight.** 1989. Purification of a set of cellular polypeptides that bind to the purine-rich cis-regulatory element of herpes simplex virus immediate early genes. Genes Dev. **3:**1372–1383.
32. **Lynas, C., K. A. Laycock, S. D. Cook, T. J. Hill, W. A. Blyth, and N. J. Maitland.** 1989. Detection of herpes simplex virus type 1 gene expression in latently and productively infected mouse ganglia using the polymerase chain reaction. J. Gen. Virol. **70:**2345–2355.
33. **Mavromara-Nazos, P., S. Silver, J. Hubenthal-Voss, J. L. C. McKnight, and B. Roizman.** 1986. Regulation of herpes simplex virus 1 genes: α gene sequence requirement for transient induction of indicator genes regulated by β or late (γ2) promoters. Virology **149:**152–164.
34. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. **69:**1531–1574.
35. **McGeoch, D. J., A. Dolan, S. Donald, and F. J. Rixon.** 1985. Sequence determination and genetic content of the short unique region in the genome of herpes simplex virus type 1. J. Mol. Biol. **181:**1–13.
36. **Mehta, A., J. Maggioncalda, O. Bagasra, S. Thikkavarapu, and P. Saikumari.** 1995. In situ DNA PCR and RNA hybridization detection of herpes simplex virus sequences in trigeminal ganglia of latently infected mice. Virology **206:**633–640.
37. **Mellerick, D. M., and N. W. Fraser.** 1987. Physical state of the latent herpes simplex virus genome in a mouse model system: evidence suggesting an episomal state. Virology **158:**265–275.
38. **Minagawa, H., S. Tanaka, Y. Toh, and R. Mori.** 1994. Detection of herpes simplex virus type 1-encoded RNA by polymerase chain reaction: different pattern of viral RNA detection in latently infected murine trigeminal ganglia following in vitro or in vivo reactivation. J. Gen. Virol. **75:**647–650.
39. **Mosca, J. D., D. P. Bednarik, N. B. K. Raj, C. A. Rosen, J. G. Sodroski, W. A. Haseltine, G. S. Hayward, and P. M. Pitha.** 1987. Activation of human immunodeficiency virus by herpesvirus infection: identification of a region within the long terminal repeat that responds to a transacting factor encoded by herpes simplex type 1. Proc. Natl. Acad. Sci. USA **84:**7408–7412.
40. **Muggeridge, M. I., and N. W. Fraser.** 1986. Chromosomal organization of the herpes simplex virus genome during acute infection of the mouse central nervous system. J. Virol. **59:**764–767.
41. **Nichol, P. F., J. Y. Chang, E. M. Johnson, Jr., and P. D. Olivo.** 1996. Herpes simplex virus gene expression in neurons: viral DNA synthesis is a critical regulatory event in the branch point between lytic and latent pathways. J. Virol. **70:**5476–5486.
42. **O'Hare, P., and C. R. Goding.** 1988. Herpes simplex virus regulatory elements and the immunoglobulin octamer bind a common factor and are both targets for virion transactivation. Cell **52:**435–445.
43. **O'Hare, P., and G. S. Hayward.** 1985. Evidence for a direct role for both the 175,000- and 110,000-molecular-weight immediate-early proteins of herpes simplex virus in the transactivation of delayed-early promoters. J. Virol. **53:**751–760.
44. **O'Hare, P., and G. S. Hayward.** 1985. Three *trans*-acting regulatory proteins of herpes simplex virus modulate immediate-early gene expression in a pathway involving positive and negative feedback regulation. J. Virol. **56:** 723–733.
45. **Rafield, L. F., and D. M. Knipe.** 1984. Characterization of the major mRNAs transcribed from the genes for glycoprotein B and DNA binding protein ICP8 of herpes simplex virus type 1. J. Virol. **49:**960–969.
46. **Ralph, W. M. J., M. S. Cabatingan, and P. A. Schaffer.** 1994. Induction of herpes simplex virus type 1 immediate-early gene expression by a cellular activity expressed in Vero and NB41A3 cells after growth arrest-release. J. Virol. **68:**6871–6882.
47. **Ramakrishnan, R., M. Levine, and D. J. Fink.** 1994. PCR-based analysis of herpes simplex virus type 1 latency in the rat trigeminal ganglion established with a ribonucleotide reductase-deficient mutant. J. Virol. **68:**7083–7091.
48. **Ramakrishnan, R., P. L. Poliani, M. Levine, J. C. Glorioso, and D. J. Fink.** 1996. Detection of herpes simplex virus type 1 latency-associated transcript expression in trigeminal ganglia by in situ reverse transcriptase PCR. J. Virol. **70:**6519–6523.
49. **Randazzo, B. P., S. Kesari, R. M. Gesser, D. Alsop, J. C. Ford, S. M. Brown, A. R. MacLean, and N. W. Fraser.** 1995. Treatment of experimental intracranial murine melanoma with a neuro-attenuated herpes simplex virus-1 mutant. Virology **211:**94–101.
50. **Rock, D. L., and N. W. Fraser.** 1983. Detection of HSV-1 genome in the central nervous system of latently infected mice. Nature (London) **302:**523–525.
51. **Roizman, B., and A. E. Sears.** 1996. Herpes simplex viruses and their replication, p. 2231–2296. *In* B. N. Fields and D. M. Knipe (ed.), Fundamental virology. Raven Press, Ltd., New York, N.Y.
52. **Ryder, K., and D. Nathans.** 1988. Induction of protooncogene c-jun by serum growth factors. Proc. Natl. Acad. Sci. USA **85:**8464–8467.
53. **Sears, A. E., V. Hukkanen, M. A. Labow, A. J. Levine, and B. Roizman.** 1991. Expression of the herpes simplex virus 1 α transinducing factor (VP16) does not induce reactivation of latent virus or prevent the establishment of latency in mice. J. Virol. **65:**2929–2935.
54. **Showalter, S. D., M. Zweig, and B. Hampar.** 1981. Monoclonal antibodies to herpes simplex virus type 1 proteins, including the immediate-early protein ICP 4. Infect. Immun. **34:**684–692.
55. **Smythe, W. R., H. C. Hwang, K. M. Amin, S. J. Eck, J. M. Wilson, L. R. Kaiser, and S. M. Albelda.** 1994. Recombinant adenovirus transfer of the HSV-thymidine kinase gene to thoracic neoplasms: an effective in vitro drug sensitization system. Cancer Res. **54:**2055–2059.
56. **Spivack, J. G., and N. W. Fraser.** 1987. Detection of herpes simplex type 1 transcripts during latent infection in mice. J. Virol. **61:**3841–3847.
57. **Spivack, J. G., G. M. Woods, and N. W. Fraser.** 1991. Identification of a novel latency-specific splice donor signal within the herpes simplex virus type 1 2.0-kilobase latency-associated transcript (LAT): translation inhibition of LAT open reading frames by the intron within the 2.0-kilobase LAT. J. Virol. **65:**6800–6810.
58. **Steiner, I., J. G. Spivack, S. L. Deshmane, C. I. Ace, C. M. Preston, and N. W. Fraser.** 1990. A herpes simplex virus type 1 mutant containing a nontransinducing Vmw65 protein establishes latent infection in vivo in the absence of viral replication and reactivates efficiently from explanted trigeminal ganglia. J. Virol. **64:**1630–1638.
59. **Stepchenko, A. G.** 1992. The nucleotide sequence of mouse Oct-1 cDNA. Nucleic Acids Res. **20:**1419.
60. **Stevens, J. G., and M. L. Cook.** 1971. Latent herpes simplex virus in spinal ganglia of mice. Science **173:**843–845.
61. **Stow, N. D., E. C. McMonagle, and A. J. Davison.** 1983. Fragments from both termini of the herpes simplex virus type 1 genome required for the encapsidation of viral DNA. Nucleic Acids Res. **11:**8205–8220.
62. **Trojanowski, J., R. Mantione, J. Lee, D. Seid, T. You, L. Inge, and V. Lee.** 1993. Neurons derived from a human teratocarcinoma cell line establishes molecular and structural polarity following transplantation into the rodent brain. Exp. Neurol. **122:**283–294.
63. **Valyi-Nagy, T., S. L. Deshmane, A. J. Dillner, and N. W. Fraser.** 1991. Induction of cellular transcription factors in trigeminal ganglia of mice by corneal scarification, herpes simplex virus type 1 infection, and explantation of trigeminal ganglia. J. Virol. **65:**4142–4152.
64. **Watson, R. J., C. M. Preston, and J. B. Clements.** 1979. Separation and characterization of herpes simplex virus type 1 immediate-early mRNAs. J. Virol. **31:**42–52.
65. **Wong, H., W. D. Anderson, T. Cheng, and K. T. Riabowol.** 1994. Monitoring mRNA expression by the polymerase chain reaction: the "primer dropping" method. Anal. Biochem. **223:**251–258.
66. **Wroblewska, Z., K. Savage, J. G. Spivack, and N. W. Fraser.** 1989. Detection of HSV-1 proteins prior to the appearance of infectious virus in mouse trigeminal ganglia during reactivation of latent infection. Virus Res. **14:**95–106.
67. **Yao, F., and P. A. Schaffer.** 1995. An activity specified by the osteosarcoma line U2OS can substitute functionally for ICP0, a major regulatory protein of herpes simplex virus type 1. J. Virol. **69:**6249–6258.