

Evidence for a Novel Regulatory Pathway for Herpes Simplex Virus Gene Expression in Trigeminal Ganglion Neurons

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Thymidine kinase (TK)-negative (TK⁻) mutant strains of herpes simplex virus type 1 (HSV-1) show reduced expression of α and β viral genes during acute infection of trigeminal ganglion neurons following corneal infection (M. Kosz-Vnenchak, D. M. Coen, and D. M. Knipe, *J. Virol.* 64:5396-5402, 1990). It was surprising that a defect in a β gene product would lead to decreased α and β gene expression, given the regulatory pathways demonstrated for HSV infection of cultured cells. In this study, we have examined viral gene expression during reactivation from latent infection in explanted trigeminal ganglion tissue. In explant reactivation studies with wild-type virus, we observed viral productive gene expression over the first 48 h of explant incubation occurring in a temporal order (α , β , γ) similar to that in cultured cells. This occurred predominantly in latency-associated transcript-positive neurons but was limited to a fraction of these cells. In contrast, TK⁻ mutant viruses showed greatly reduced α and β gene expression upon explant of latently infected trigeminal ganglion tissue. An inhibitor of viral TK or an inhibitor of viral DNA polymerase greatly decreased viral lytic gene expression in trigeminal ganglion tissue latently infected with wild-type virus and explanted in culture. These results indicate that the regulatory mechanisms governing HSV gene expression are different in trigeminal ganglion neurons and cultured cells. We present a new model for viral gene expression in trigeminal ganglion neurons with implications for the nature of the decision process between latent infection and productive infection by HSV.

The mechanisms that govern the choice between infection pathways leading to productive infection by herpes simplex virus (HSV) versus a nonproductive latent infection remain to be elucidated. The regulatory pathways during productive infection of cultured cells have been studied extensively. Initially after infection, the α (immediate-early) gene products are expressed. The products of these genes, in particular ICP4, activate and regulate the expression of later viral genes. The next class of gene products, the β (delayed-early) gene products, is largely involved in replication of viral DNA. Following viral DNA replication, expression of γ (late) gene products is maximal (26, 47). The α gene products ICP4 (8, 39, 64) and ICP27 (35, 44, 48, 53, 60) have been reported to down regulate α gene expression in infected cells. The β gene products (20), in particular ICP8 (14-16), have been reported to down regulate α , β , and, under certain conditions, γ gene expression. Viral DNA replication or late gene products also play a role in down regulation of α and β gene expression in that inhibitors of viral DNA replication in cultured cells lead to sustained α and β gene expression (5). Relevant to this study in which viral transcripts have been examined, α transcripts accumulate in cultured cells to normal levels in the presence of viral DNA synthesis inhibitors (18), and β mRNAs overaccumulate in the presence of viral DNA synthesis inhibitors (18, 42).

In contrast, during latent infection of neurons *in vivo*, the

only viral gene product expressed abundantly is the latency-associated transcript (LAT) (6, 59). Although the precise role of LAT is not known, this transcript or gene products encoded by it promote reactivation from latency (19, 29, 58, 62) and possibly establishment of latency (49).

Two important issues regarding establishment of latent infection are the stage in infection when the latent versus production pathways diverge and the role of viral or host gene products in affecting this choice of infection pathways. Regarding the first issue, mutant viruses (24, 27, 63) or wild-type (wt) virus, at least in some cells (32, 52, 54), is capable of establishing latent infections without complete replication or even substantial viral gene expression in neurons. This has been interpreted to mean that the latent infection pathway deviates very early from the productive infection pathway (27, 32, 63). However, the *in situ* hybridization techniques used in many of these studies are not sensitive enough to distinguish between the total absence of viral gene expression and low levels of viral gene expression during establishment of latent infection. In the latter case, the productive infection could be aborted at an early stage after limited viral gene expression. Regarding the second issue, all viral mutants tested thus far are capable of establishing latent infection (4, 9, 24, 29, 31, 38, 51, 61), and thus there is no evidence that expression of any viral gene product is required for latency. On this basis, the possibility that the neuron controls the establishment of latency has been raised (24, 32). Nevertheless, it is conceivable that viral gene products could participate in or influence the choice of infection pathways, although none are required for establishment of latency.

Thymidine kinase (TK)-negative (TK⁻) mutant viruses

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replicate at the site of inoculation in mice, can enter sensory neurons and establish latency, but fail to reactivate (4, 9, 31, 61). TK⁻ viruses have been shown to replicate poorly in growth-arrested cells in culture (10, 22). HSV TK is believed to provide deoxyribonucleoside triphosphate precursors for viral DNA replication in resting cells, in which the host enzyme would not be expressed. Therefore, the defect in replication in resting cells for TK⁻ mutant viruses would be at the level of viral DNA replication. It was surprising to observe, therefore, that α and β viral gene expression is virtually nondetectable during acute infection of trigeminal ganglion (TG) neurons (27), given that inhibition of viral DNA synthesis normally does not decrease levels of α and β transcripts (18, 42).

To explain these results and further define the factors regulating viral gene expression in TG neurons, we have examined viral gene expression in TG tissue during reactivation upon tissue explant. We observed that inhibition of TK activity or inhibition of viral DNA replication severely decreases α and β gene expression. From these results, we postulate a new regulatory pathway for viral gene expression in TG neurons.

MATERIALS AND METHODS

Viruses. The wt HSV-1 KOS strain and the TK⁻ mutant virus strain *dl*sactk have been described previously (4, 27).

Infection of mice and in vitro reactivation procedures. Thirty days after corneal inoculation of mice (28), trigeminal ganglia were removed from the animals as described previously (28), cut into two pieces, and explanted in culture medium at 37°C in the absence of other cells. After various times of incubation, the ganglia from each experimental group of mice were pooled, frozen, and sectioned as described by Kosz-Vnenchak et al. (27).

In situ hybridization. Methods for in situ hybridization using ³H- and ³⁵S-labeled DNA probes have been described previously (27). The DNA probes used were pIPH (29) for LAT, pK1-2 (7) for ICP4, pBH27 (43) for ICP27, p8B-S (12) for ICP8, and a clone containing the *Eco*RI-I-*Bam*HI-I DNA fragment (11, 15) for gC. For analysis, serial sections of each ganglion were prepared, and sequential sections from at least three to four locations in each ganglion were hybridized with LAT, ICP4, ICP27, ICP8, and gC gene probes.

Inhibitors. Ro 31-5140 (33, 34), kindly provided by J. A. Martin, Roche Research Centre, Welwyn Garden City, United Kingdom, was dissolved in dimethyl sulfoxide and stored at -20°C. In experiments for which addition of this drug to explant cultures is indicated, ganglia were collected in medium containing 64 μ M Ro 31-5140 and following bisection were explanted in medium containing 100 μ M Ro 31-5140. Phosphonoacetic acid (PAA) was obtained from Sigma Chemical Co.

RESULTS

Viral gene expression following explant of wild-type virus-infected ganglia. To study viral gene expression in TG neurons, we established a system to examine viral gene expression in ganglionic tissue during explant reactivation. At 30 days postinoculation, when latent infection is known to be established in the trigeminal ganglia, we removed the latently infected ganglia, cut the ganglia into two pieces, and placed them in culture medium at 37°C. After various times of incubation ranging from 12 to 48 h, the tissue pieces were pooled, placed in embedding medium, frozen, and sectioned.

The resulting sections were processed for in situ hybridization and hybridized with probes for the mRNAs encoding the productive-infection gene products ICP27, ICP8, and gC or for LAT. After 12 h of incubation, only the LAT probe showed positive hybridization; none of the productive-infection transcripts were detected (results not shown). LAT was also detected after 24 and 48 h of incubation (Fig. 1A and B and 2A). After 24 h of incubation, the probes for the α transcripts for ICP4 and ICP27 showed strong hybridization (Fig. 1C and D) while the probes for the β transcript for ICP8 showed weak hybridization (Fig. 1E; Table 1). We have defined strong hybridization as a high density of silver grains, usually too dense to allow individual grains to be distinguished (Fig. 1A to D). We have defined weak hybridization as cells exhibiting approximately 10 to 20 grains or less, a level above the background level but significantly less than the level of hybridization with other probes. After 24 h of incubation, the gC probe showed little, if any, hybridization (Fig. 1F).

After 36 h of incubation, the productive transcripts for ICP27, ICP4, and ICP8 were strongly positive, but hybridization with the gC probe was still detected only weakly (Table 1). After 48 h of incubation, the productive transcripts for ICP27, ICP4, ICP8, and gC were all strongly positive (Fig. 2B to E; Table 1). Therefore, it appeared that sequential expression of viral productive-infection gene products was occurring in a temporal order similar to that during productive infection of cultured cells (26, 47). The sequential appearance of viral gene products is likely to be occurring during reactivation in the latently infected neurons because viral DNA replication and infectious virus are first observed at approximately 48 h after explant in similar systems (56, 57) and expression of viral proteins in the reactivating neuron has been documented at 48 h postexplant (36). Others have considered reactivation to occur in the first 48 h after explant and secondary replication to occur during the next 48 h (56). Thus, we feel that it is likely that we are studying events in the reactivating neuron during the first 24 to 48 h of explant culture.

Although it was difficult to quantify precisely the number of cells exhibiting hybridization with the productive-infection probes (27), we estimated that there was an average of three positive cells per section after 48 h of incubation. In contrast, the LAT probe showed hybridization to an average of 10 neurons per section after 24 h of incubation. This decreased to approximately three LAT-positive neurons per section after 36 to 48 h of incubation, a decrease consistent with that found in a previous study (56). It appeared that not all neurons expressing LAT were undergoing reactivation in our system. Analysis of serial sections revealed that nearly all neurons showing hybridization with productive-infection probes also showed hybridization with the LAT probe (results not shown). Thus, productive infection and presumably reactivation under these conditions occurred predominantly in LAT-positive neurons but in only a subset of these neurons. The fact that neurons expressing productive transcripts of α and β genes also expressed LAT indicated that these cells were undergoing reactivation and not secondary infection because LAT is expressed as a γ gene in productively infected cells (45, 55).

Expression of viral genes in neurons infected with TK⁻ virus. Previous studies had shown that TK⁻ mutant viruses were capable of establishing a latent infection in mouse TG neurons but incapable of reactivation upon explant (4, 9, 31, 61). To examine viral gene expression under conditions in which reactivation was blocked, we used the explant reac-

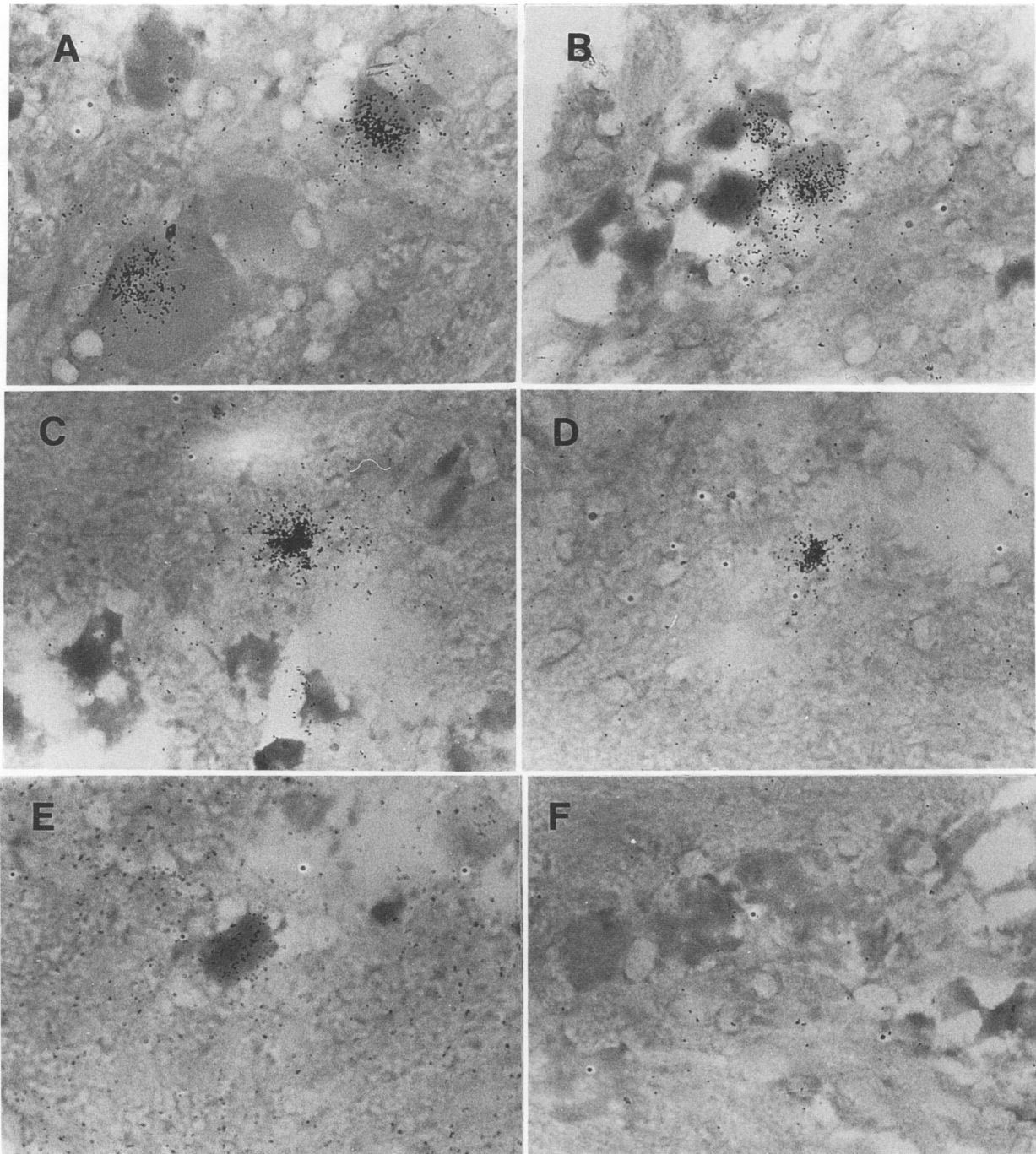


FIG. 1. Expression of viral genes during explant reactivation by wt virus. After 24 h of incubation, the ganglion pieces were pooled, frozen, sectioned, and hybridized with various probes. (A and B) LAT probe; (C) ICP27 mRNA probe; (D) ICP4 mRNA probe; (E) ICP8 mRNA probe; (F) gC mRNA probe.

tivation system described above. Mice were infected with the *Δ*tsactk TK⁻ mutant virus (4), which is reactivation negative as a result of its *tk* mutation (21); after 30 days, the trigeminal ganglia were removed and explanted as described above. Under these conditions, no reactivation was observed (21). After 48 h of incubation, the ganglion tissue was frozen and sectioned for hybridization. Although LAT hybridization was detected in a high proportion of the mutant-

infected ganglia, little or no hybridization was observed with the productive-infection probes (Fig. 3B to D; Table 2). In a few ganglia, weak, limited hybridization was observed over a few cells (Fig. 4B; Table 2). Similar results were observed with tissues incubated for 12 or 24 h (results not shown). Thus, expression of these productive-infection genes, likely in reactivating neurons as described above, was greatly reduced in neurons latently infected with TK⁻ mutant virus

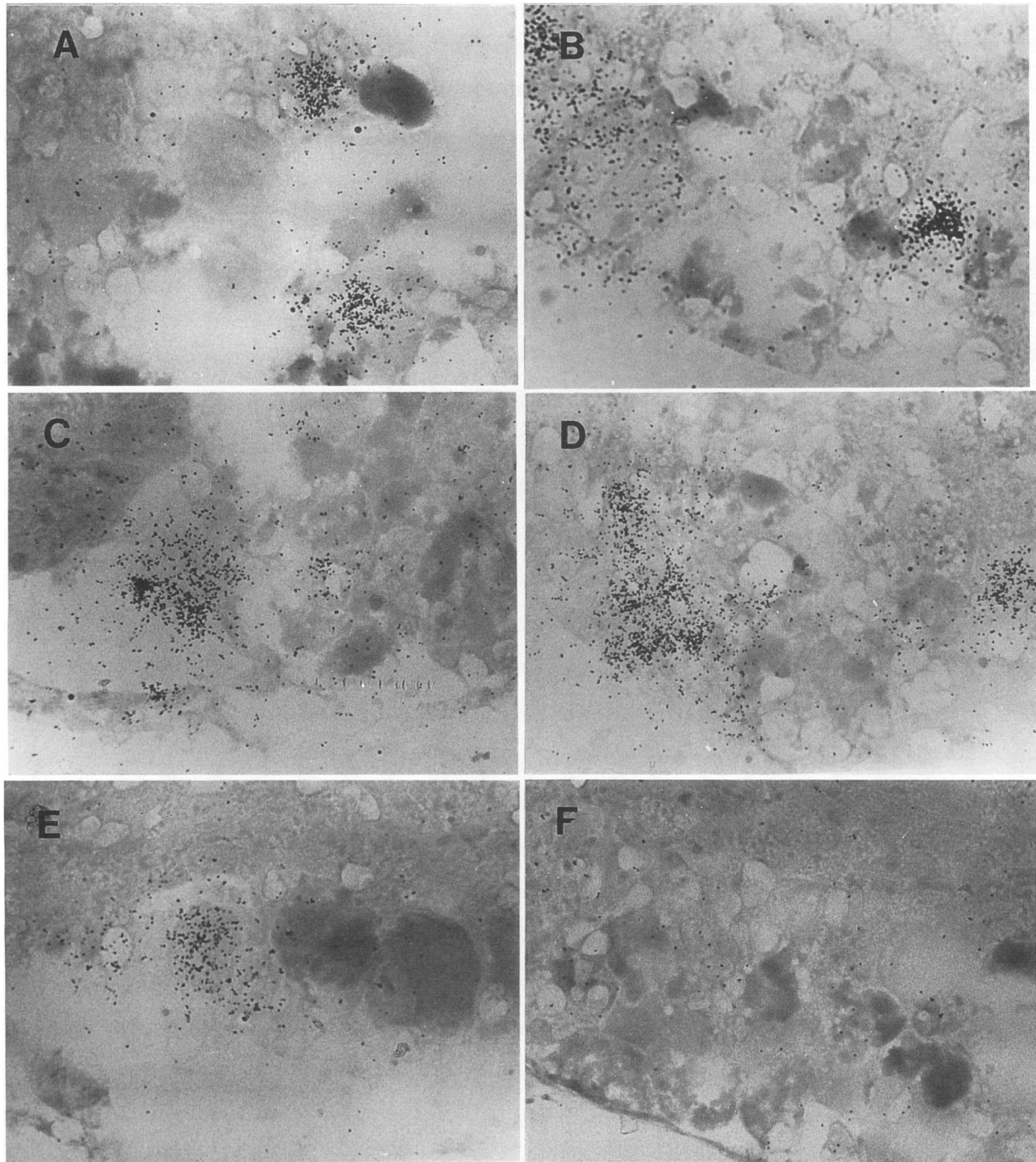


FIG. 2. Viral gene expression after 48-h incubation of TG tissue. (A) LAT probe; (B) ICP27 mRNA probe; (C) ICP4 mRNA probe; (D) ICP8 mRNA probe; (E) gC mRNA probe; (F) uninfected mouse ganglion tissue probed with the LAT probe.

and incubated under conditions for explant reactivation. The reduced expression of productive gene products under reactivation conditions was similar to the greatly reduced viral gene expression during establishment of latent infection by TK⁻ mutant viruses (27).

Viral gene expression during incubation of tissue with a TK inhibitor. Compounds that inhibit HSV TK activity have been shown to inhibit reactivation under explant conditions (1, 21, 30). Use of these compounds provides a way to

TABLE 1. Viral gene expression during reactivation of wt virus

Incubation time (h)	Fraction of ganglia positive with probe for:				
	LAT	ICP4	ICP27	ICP8	gC
24	12/12	6/8	8/12	4 ^a /12	2 ^a /12
36	4/4	ND ^b	2/4	2/4	2 ^a /4
48	13/15	5/6	13/15	13/15	13/15

^a Weak hybridization, as defined in the text.

^b ND, not determined.

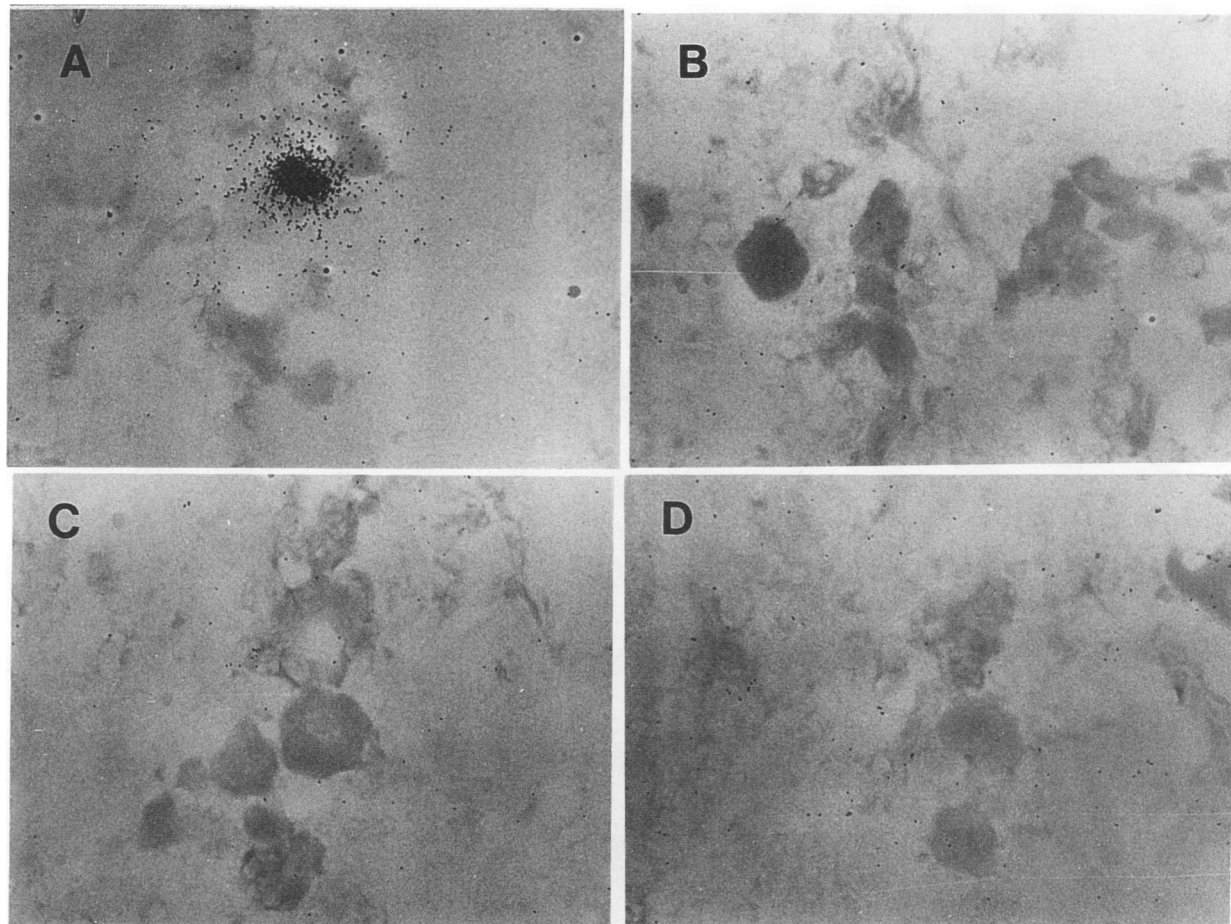


FIG. 3. Viral gene expression by TK⁻ mutant virus after 48-h incubation in vitro. (A) LAT probe; (B) ICP27 mRNA probe; (C) ICP8 mRNA probe; (D) gC mRNA probe.

examine reactivation of latent wt virus in which TK enzyme activity is inhibited. To determine whether a TK-inhibitory drug caused a similar lack of gene expression as a genetic defect in TK expression, we incubated ganglia latently infected with wt virus in the presence of an inhibitor of the HSV TK enzyme, Ro 31-5140 (33, 34), under conditions that significantly inhibit reactivation (21). Incubation of ganglia with this drug also greatly reduced the expression of lytic transcripts for ICP27, ICP8, and gC (Fig. 5B to D; Table 3). Thus, two separate methods for removing TK activity from an infected ganglion resulted in the same effect, i.e., inhibition of expression of all productive-infection genes. These results make it likely that the effect on viral gene expression was due to the alterations in TK and not due to secondary mutations in the mutant viruses or to secondary effects of the

inhibitory drug. These results also indicated that the reduced gene expression by TK⁻ viruses was not likely to be due to infection of different neurons or different types of infection by TK⁻ viruses because these latent infections involved infections by wt virus.

Viral gene expression during reactivation in the presence of a viral DNA synthesis inhibitor. Two major hypotheses remained to explain the effect of reduction of viral gene expression in the presence of reduced TK levels. First, TK is believed to provide deoxynucleotide precursors for viral DNA synthesis, and lack of TK would be predicted to result in a lack of viral DNA synthesis in resting cells. Therefore, the effect of alteration of gene expression could be the result of a lack of viral DNA synthesis. Alternatively, TK could be directly involved in regulating viral gene expression in TG neurons. To test the first hypothesis, we determined whether a known inhibitor of viral DNA synthesis, PAA, could affect viral gene expression in the reactivation system. Ganglia latently infected with wt virus were explanted in the presence of 300 µg of PAA per ml to inhibit viral DNA synthesis. After 48 h, the ganglia were analyzed for expression of productive-infection transcripts. Ganglia treated with PAA showed greatly reduced or no detectable expression of productive gene transcripts, although LAT expression was detected in all ganglia (Table 4). Thus, inhibition of viral DNA synthesis with PAA decreased α and β viral gene

TABLE 2. Viral gene expression during incubation of TK⁻ mutant-infected tissue

Virus	Fraction of ganglia positive with probe for:				
	LAT	ICP4	ICP27	ICP8	gC
KOS wt	7/8	3/4	7/8	7/8	7/8
<i>dlsac</i> TK ⁻	7/10	2 ^a /4	1 ^a /10	0/10	1 ^a /10

^a Weak hybridization.

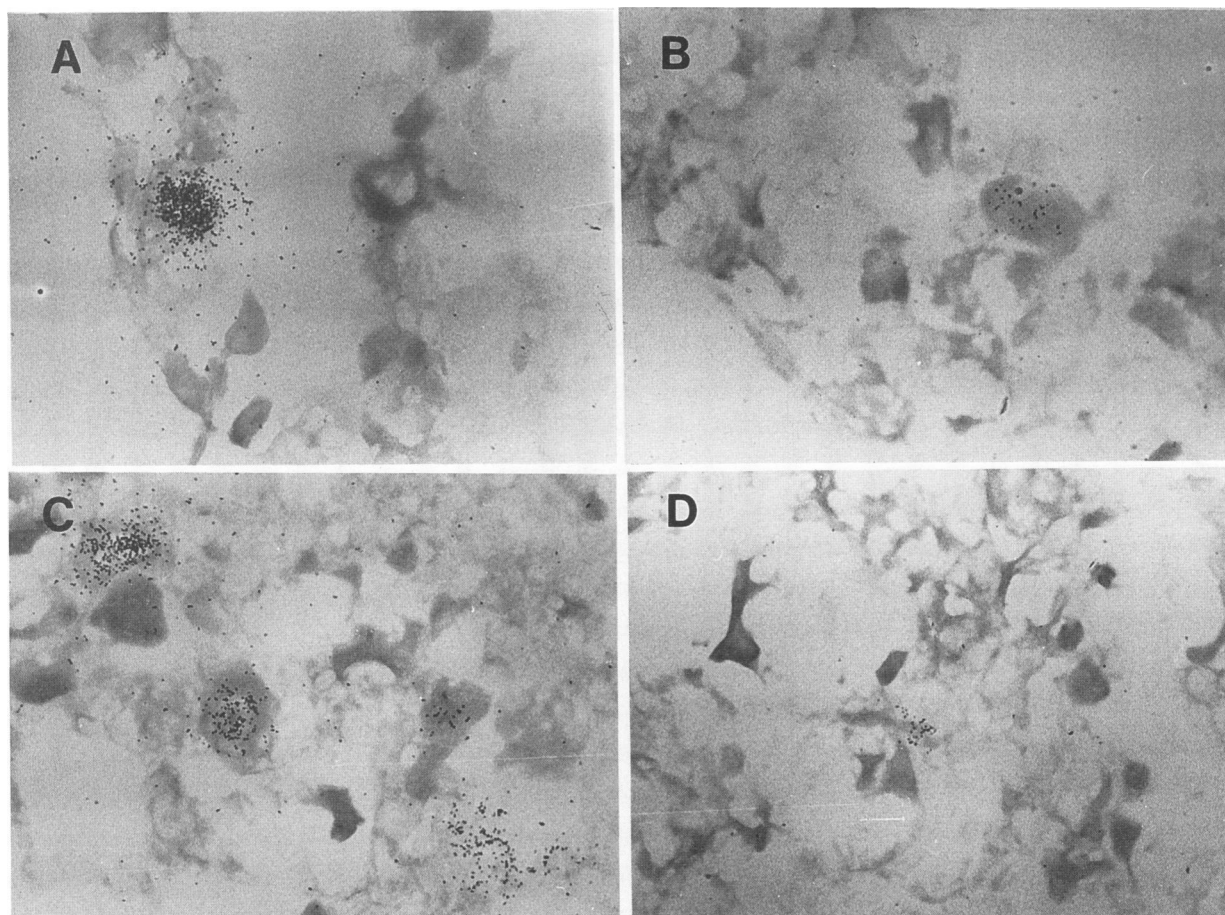


FIG. 4. Examples of weak hybridization. (A) Strong hybridization in *d/sacTK* mutant-infected tissue probed with the LAT probe; (B) weak hybridization in *d/sacTK* mutant-infected tissue probed with the ICP27 mRNA probe; (C) strong hybridization in wt virus-infected tissue incubated with Ro 31-5140; (D) weak hybridization in wt virus-infected tissue incubated with Ro 31-5140 and probed with the ICP27 mRNA probe.

expression in TG neurons, similar to the situations in which TK activity was absent.

The effect of PAA might be due to toxic effects of the inhibitor, although the expression of LAT in all ganglia argued against this explanation. To control for toxic effects of PAA, we examined the ability of the viral mutant PAA⁵, which is resistant to PAA as a result of a DNA polymerase gene mutation (3), to reactivate in the presence of 300 μ g of PAA per ml. Under these conditions, 100% (six of six) of the PAA⁵-infected ganglia showed reactivation. Therefore, the effects of PAA were due to its inhibition of the viral DNA polymerase and not to other toxic effects of the drug. The inhibition of viral productive gene expression in the neurons was likely due to inhibition of viral DNA replication.

TK⁻ virus gene expression in the mouse cornea. We examined the phenotype of the TK⁻ mutant virus in another mouse tissue to determine whether the phenotype was specific for neurons. We examined α , β , and γ viral RNA accumulation in the cornea of mice infected with *d/sactk*. At 2 days postinfection, the eyes were removed, sectioned, and hybridized with viral probes. Hybridization was observed with LAT, α , β , and γ probes (Fig. 6). Thus, the lack of viral productive transcripts by TK⁻ mutant viruses was not observed in all mouse tissues. In addition, this finding

provides evidence that the method used to detect viral gene expression can detect viral gene expression in animals infected with TK⁻ virus in tissues in which viral replication is taking place.

DISCUSSION

We have shown previously that during acute infection of TG neurons *in vivo* and establishment of latent infection, TK⁻ HSV mutant viruses express significantly reduced amounts of α and β viral gene products (27). This finding was paradoxical for two reasons. First, TK is a β viral gene product, and according to the lytic regulatory cascade (26, 47), lack of a β gene product should not reduce expression of α and β viral gene products. Second, TK is believed to play a role in providing deoxyribonucleoside triphosphate precursors for viral DNA replication in resting cells such as neurons, so lack of HSV TK should reduce viral DNA replication. In cultured cells inhibition of HSV DNA replication does not decrease levels of α or β gene transcripts (18, 42); thus, the TK⁻ mutant viruses showed a novel phenotype in TG neurons.

To explain these results, we proposed several possible explanations (27): the mechanisms controlling viral gene

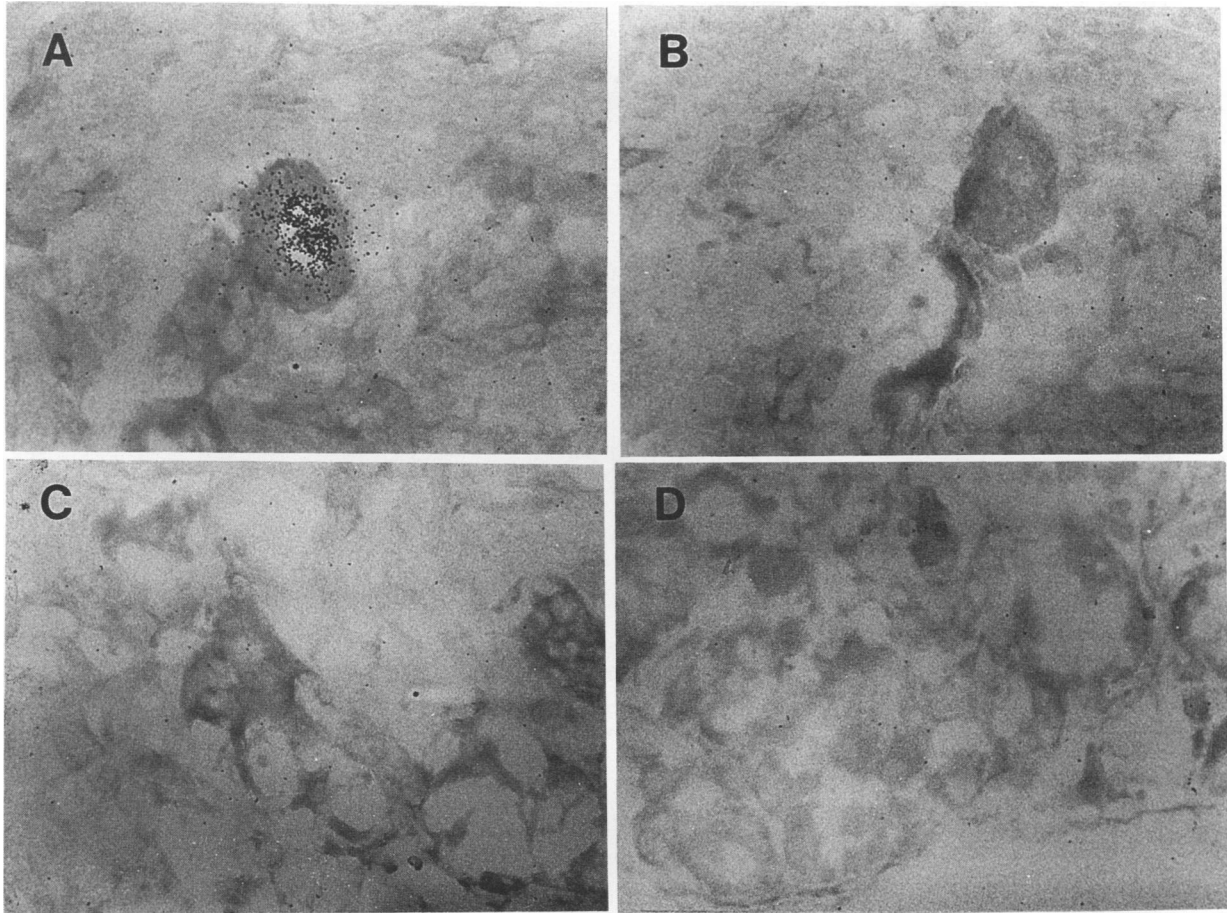


FIG. 5. Viral gene expression by wt virus after 48-h incubation of trigeminal ganglia in vitro with the TK inhibitor Ro 31-5140. (A) LAT probe; (B) ICP27 mRNA probe; (C) ICP8 mRNA probe; (D) gC mRNA probe.

expression are different in neurons as opposed to other cells (model 1); the TK⁻ phenotype leads to reduced multiplicity of infection of neurons (model 2); the spread of TK⁻ mutant viruses or the type of neuron that they infect is different (model 3); and the lytic transcripts observed with wt-infected ganglia are actually transcripts from both strands of most of the genome observed at late times of infection in nuclear runoff assays (16, 65) (model 4). We do not currently entertain model 4 because we have not observed extensive genomic transcription at late times, using pulse-labeling of RNA in intact cells (23). The nuclear runoff technique appears to give artifactual symmetric transcription at late times after HSV infection. Thus, we consider further only the first three models in this report.

TABLE 3. Gene expression during incubation of tissue with the TK inhibitor Ro 31-5140

Infection conditions	Fraction of ganglia positive with probe for:			
	LAT	ICP27	ICP8	gC
wt virus	7/9	8/9	8/9	8/9
wt virus + 100 μ M Ro 31-5140	10/13	1 ^a /13	2 ^a /13	1 ^a /11

^a Weak hybridization.

We have performed the experiments in this study in a related but different system, i.e., reactivation of virus from latent infection by ganglion tissue explant, because this type of system allowed examination of regulatory mechanisms affecting HSV gene expression in a situation in which the experimental conditions could be altered, such as the addition of compounds that inhibit specific viral enzymes. The phenotype of TK⁻ HSV mutant strains demonstrating a lack of lytic viral gene expression in TG neurons was observed both during acute infection and establishment of latent infection and during reactivation from latent infection, and this finding argues that at least some similar mechanisms are operating in the two situations. For this reason, we believe that the results from this reactivation system can be extrapolated to establishment of latent infection in TG neurons.

TABLE 4. Viral gene expression during incubation of tissue with PAA

Incubation conditions	Fraction of ganglia positive with probe for:			
	LAT	ICP27	ICP8	gC
wt virus	3/3	3/3	3/3	3/3
wt virus + PAA	8/8	1 ^a /8	0/8	0/8

^a Weak hybridization.

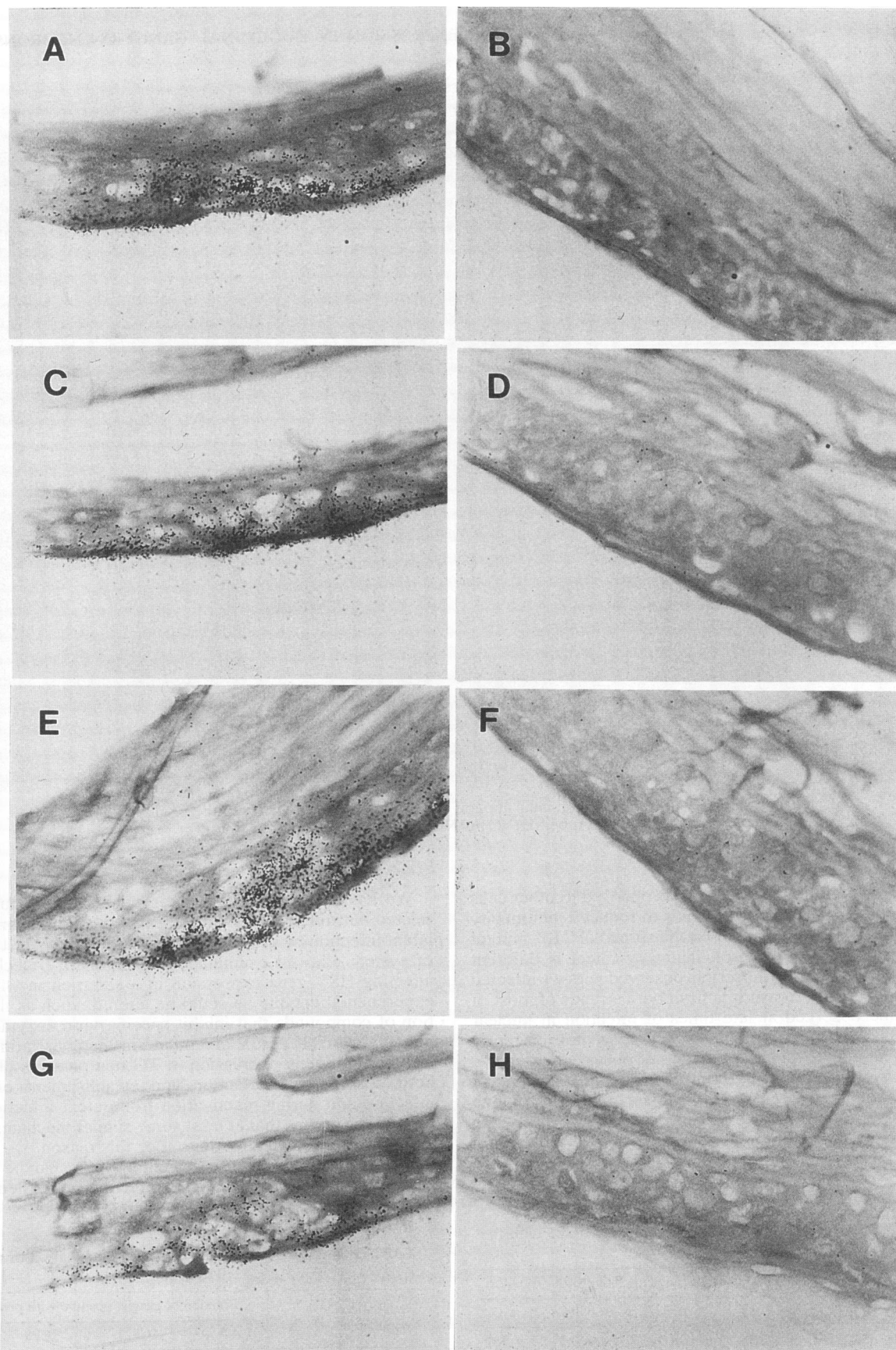


FIG. 6. Viral gene expression in corneal tissues infected with *dsacTK*. At 3 days after corneal scarification and viral inoculation, the mouse eyes were removed, frozen, sectioned, and hybridized with viral probes. (A) *dsacTK* infection, LAT probe; (B) mock infection, LAT probe; (C) *dsacTK* infection, ICP27 mRNA probe; (D) mock infection, ICP27 mRNA probe; (E) *dsacTK* infection, ICP8 mRNA probe; (F) mock infection, ICP8 mRNA probe; (G) *dsacTK* infection, gC mRNA probe; (H) mock infection, gC mRNA probe.

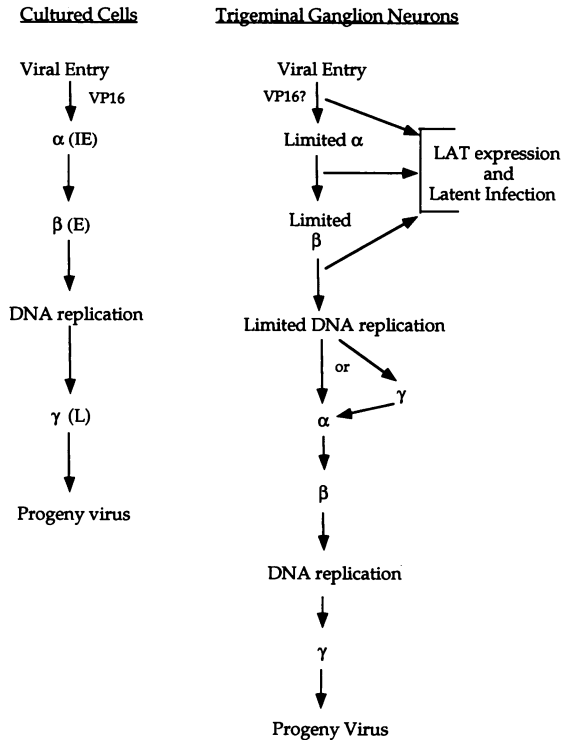


FIG. 7. Pathways of HSV gene activation. The scheme illustrates regulatory pathways for viral gene activation in the two situations. Possible negative regulatory pathways as summarized in the introduction are not illustrated. IE, immediate early; E, early; L, late.

We observed that addition of inhibitors of viral TK or viral DNA polymerase inhibited viral α and β gene expression in TG neurons latently infected with wt virus. This finding rules out models 2 and 3 described above because diminished lytic gene expression was observed with neurons latently infected with wt virus in these experiments. This means that some unusual feature of TG neuron infection by the TK⁻ virus is not needed to observe the diminished lytic gene expression. We therefore believe that model 1, an altered regulatory network in TG neurons, is most likely to explain this novel phenotype. To explain how inhibition of viral TK or viral DNA replication decreases viral lytic gene expression, we propose a model for viral gene expression in TG neurons which has implications for the nature of the switch between lytic and latent infection by HSV (see below).

Although we have investigated only TG neurons, a similar effect may have been seen during infection of rat sympathetic neuron cultures derived from cervical ganglia (66). These investigators observed that optimal survival of the cultures and establishment of *in vitro* latency were observed with cultures incubated with acyclovir, an inhibitor of viral DNA replication, during infection. Expression of α genes during acute infection of these cultures in the presence or absence of acyclovir has not been examined, but if these neurons are similar to TG neurons, reduced α gene expression would be expected.

A model for regulation of HSV gene expression in TG neurons. From our results in this system, we propose the following new model for HSV gene regulation in neurons (Fig. 7). Upon infection of neurons, α gene transcription is limited, possibly because of the lack of host cell transcrip-

tion factors that bind VP16 (50) or otherwise promote α gene transcription or the presence of inhibitors of α gene transcription (25). The low level of α proteins expressed may transactivate low-level expression of β gene products. If insufficient amounts of α or β gene products are expressed, the productive infection could be aborted and latency could be established. If a sufficient amount of viral DNA replication can occur, α gene transcription is enhanced, possibly by the mechanisms described below. Once α gene expression has been augmented, the usual cascade of HSV infection occurs, involving expression of β genes, viral DNA replication, and expression of γ genes. By this model, any means of inhibition of viral DNA replication in neurons, e.g., lack of viral TK or presence of a viral DNA polymerase inhibitor, would cause limited expression of α and β gene expression, possibly levels too low to be detected consistently by *in situ* hybridization. Further studies using more sensitive methods such as reverse transcriptase-polymerase chain reaction are under way to test this aspect of the model.

An important feature of this model is that the lytic, productive-infection pathway can be aborted at any point up to viral DNA replication to lead to a latent infection. Limited α and β viral gene expression may occur in neuronal cells in which a latent infection is eventually established. Low-level expression of α and β genes may even occur during latent infection (17).

There are several additional implications of this model. (i) Other early events in addition to transcriptional regulation of α genes could also comprise part of the decision to establish a latent or productive infection. The ability to transactivate β genes, the ability of viral gene products such as ICP4 (8, 13, 39, 40, 64) or ICP8 (14–16) to down regulate viral gene expression, or the ability to replicate viral DNA replication could contribute to this decision. (ii) Viral gene products, in particular α or β gene products, could play a role in regulating the decision to establish a latent or a productive infection. This would mean that the virus does not merely play a passive role during establishment of latent infection. In this regard, it is of interest that transcripts from the varicella-zoster virus DNA-binding protein gene (HSV ICP8 homolog) have been reported to be expressed in cells latently infected with varicella-zoster virus (37). HSV ICP8 has been shown to exert negative effects on transcription from parental viral genomes (14–16). (iii) The ultimate trigger for a full productive infection cycle or viral reactivation in neurons would be replication of viral DNA to a critical level. This aspect resembles one part of the model of Roizman and Sears (46), but they postulated that viral DNA amplification will occur during the maintenance phase of latency and “is fulfilled by a host polymerase.” In contrast, we postulate that viral DNA amplification occurs during acute infection or reactivation and is achieved by the normal productive-infection pathway occurring on a limited scale.

Possible mechanisms of the requirement for viral DNA synthesis. Optimal viral α and β gene expression in TG neurons requires viral DNA replication. This effect could be mediated by any of several mechanisms. Viral DNA replication may provide an amplified or altered template for transcription. This may reflect the poor utilization of α promoters in neuronal cells. Several hypotheses have been advanced to explain the low activity of α promoters in neurons as described above. Amplification of the viral genome could make α promoters work more efficiently in the presence of limiting transcription factors by altering the form of DNA or increasing its concentration to promote binding of

transcription factors. Alternatively, it could titrate out or release any inhibitory factors binding to parental viral DNA.

Alternatively, viral DNA replication may activate expression of a γ gene product that feeds back to activate α gene expression. A candidate molecule would be VP16, a late gene product known to activate α gene expression (2, 41). Constitutive expression of VP16 in transgenic mice was reported to not affect the ability of the virus to establish latent infection (50). VP16 may not be sufficient to disrupt latency but may still play a role in regulating viral gene expression in neurons. Other γ gene products might also function as activators of α gene expression.

Predictions of this model. Our model (Fig. 7) predicts that low-level expression of α and β genes may occur during acute TG infection or in explanted TG tissue from animals infected with TK⁻ mutant viruses. Experiments using more sensitive techniques to detect expression of viral gene products under these conditions are under way. In addition, viral mutants with alterations in other α or β gene products may show novel phenotypes with regard to expression of lytic gene products or ability to establish latency in TG neurons. This type of genetic analysis may provide information about the role of viral gene products in influencing the decision between latent and lytic infection pathways.

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