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The Equine Herpesvirus 1 (EHV-1) UL3 Gene, an ICP27 Homolog, Is Necessary for Full Activation of Gene Expression Directed by an EHV-1 Late Promoter

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We have previously reported that the equine herpesvirus 1 (EHV-1) *Xba*I G restriction fragment (nucleotides 1436 to 7943 relative to the left terminus of the EHV-1 genome [Kentucky A strain]) is required in combination with the EHV-1 immediate-early (IE) gene to achieve significant activation of two representative EHV-1 late promoter-chloramphenicol acetyltransferase (CAT) recombinants in transient expression assays. In this report, we demonstrate that the *Xba*I G-encoded UL3 gene (an ICP27 homolog) provides a *trans*-acting factor which acts (in combination with the EHV-1 IE gene product) to increase reporter gene expression directed by an EHV-1 late promoter-CAT recombinant plasmid. We show that cloned copies of UL3 can successfully substitute for the *Xba*I G fragment in CAT assays and that stop codon insertion within the UL3 open reading frame inhibits the ability of UL3 to activate reporter gene expression in *trans*.

Equine herpesvirus 1 (EHV-1), a member of the subfamily *Alphaherpesvirinae*, provides a model system with which to study various aspects of herpesvirus molecular biology and pathogenesis (2, 5, 7, 21, 26, 27). A cascade model of sequential, coordinate viral gene expression has been established for EHV-1 lytic infection in which viral gene expression may be divided into three temporal classes, designated immediate-early (IE), early, and late (4, 9). The IE class consists of a single member, termed the IE gene (map units 0.78 to 0.82 and 0.96 to 1.00) (10). The IE gene has been extensively characterized at the DNA, RNA, and protein levels (3, 11, 13, 14, 25). DNA sequence analysis has revealed that two highly conserved (>50% homology) protein domains are common to the EHV-1 IE gene product and at least two other major regulatory factors of other alphaherpesviruses, namely, ICP4 of herpes simplex virus type 1 (HSV-1) and the ORF62 gene product of varicella-zoster virus (11). In a functional characterization of the EHV-1 IE gene product, we reported that the IE gene encodes an autoregulatory factor capable of both *trans* activation and *trans* repression of target gene expression (32). The EHV-1 IE gene product alone was sufficient to achieve an approximately 60-fold activation of gene expression from a reporter plasmid bearing a representative EHV-1 early promoter (thymidine kinase promoter) linked to chloramphenicol acetyltransferase (CAT) coding sequences and a >15-fold activation of gene expression from an HSV-1 thymidine kinase-CAT construct (32). In contrast, two representative late promoter constructs bearing promoter elements of the EHV-1 glycoprotein D (gD) (1, 8) or the EHV-1 IR5 (HSV-1 US10 homolog) (17) genes linked to CAT were only marginally activated (two- to threefold) in the presence of the IE gene product. It was observed, however, that cotransfection of the EHV-1 *Xba*I G restriction fragment with the EHV-1 IE gene expression vector greatly enhanced gene expression from the gD and IR5 promoter-CAT recombinants. In this report, we identify the gene within *Xba*I G which cooperates with the IE protein to fully activate a representative EHV-1 late promoter-CAT recombinant.

The relative location of the EHV-1 *Xba*I G restriction fragment (map units 0.01 to 0.06), the arrangement of genes within *Xba*I G, and a series of *Xba*I G subclones are shown in Fig. 1. DNA sequencing and transcription mapping have revealed that the *Xba*I G fragment contains three intact genes (termed UL2, UL3, and UL4), as well as portions of two genes (UL1 and UL5) that extend beyond the boundaries of *Xba*I G (15, 16, 35, 36). The gene arrangement within this portion of the EHV-1 genome has recently been confirmed by Telford et al. (34), who have sequenced the entire genome of the Ab4 strain of EHV-1. UL2 has the potential to encode a 200-amino-acid (200-aa) protein homologous to the HSV-1 UL55 gene product (35). UL3 encodes a 470-aa protein showing 32% homology to the UL54 (ICP27) gene product of HSV-1, while UL4 encodes a 343-aa protein homologous to the UL53-encoded glycoprotein of HSV-1 (36). UL1 and UL5 are homologous to varicella-zoster virus ORF2 and HSV-1 UL52, respectively (34, 35).

*Xba*I G and various subclones of *Xba*I G were tested for the ability to enhance CAT activity directed by an EHV-1 IR5 promoter-CAT recombinant plasmid (pIR5-CAT) in the presence of an EHV-1 IE gene expression vector (pSVIE) bearing the IE gene under the control of the simian virus 40 early promoter. Construction of pSVIE and a description of the transfection and CAT assay procedures have been reported previously (32). Plasmid pIR5-CAT contains promoter elements of the IR5 gene, a late gene that is homologous to US10 of HSV-1 (17), from −1113 to +13 relative to the transcription initiation site linked to the CAT gene of plasmid pCAT-Basic (Promega). L-M cells (murine fibroblasts) were transfected with 1.4 pmol of the pIR5-CAT reporter plasmid and 0.3 pmol of the appropriate effector plasmid.

As demonstrated in Table 1 and Fig. 2 (lanes 1 and 2), cotransfection of pSVIE with the pIR5-CAT reporter plasmid resulted in an approximately threefold increase in CAT

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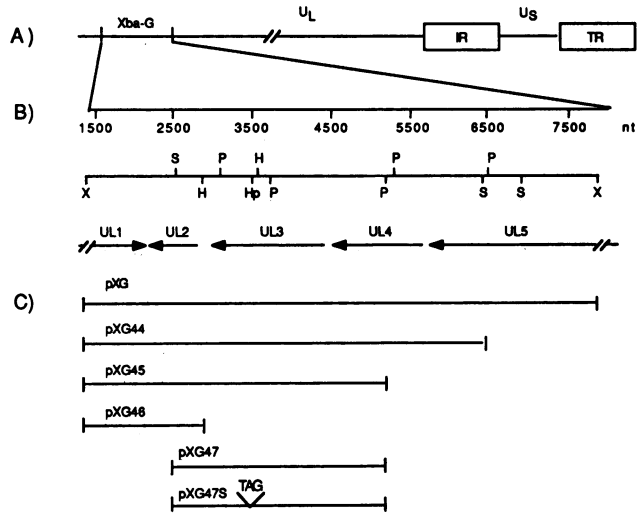

FIG. 1. Gene arrangement and subclones of the *Xba*I G restriction fragment of EHV-1. (A) The genomic position of the *Xba*I G fragment is indicated. (B) Enlargement of the *Xba*I G fragment showing the arrangement of genes within *Xba*I G and restriction enzyme sites used in the construction of *Xba*I G subclones (X, *Xba*I; S, *Sph*I; H, *Hin*dIII; P, *Pst*I; Hp, *Hpa*I). The nucleotide (nt) numbers represent distances (in base pairs) from the left terminus of the EHV-1 genome (Kentucky A strain). Arrows indicate the positions and directions of transcription of genes mapping within *Xba*I G. The UL1 and UL5 open reading frames initiate outside the boundaries of *Xba*I G. (C) Schematic representation of the cloned *Xba*I G fragment and various subclones. Abbreviations: U_L, unique long; U_S, unique short; IR, internal inverted repeat; TR, terminal repeat; TAG, stop codon linker insertion.

activity compared with basal levels of CAT activity associated with pIR5-CAT in the absence of effector plasmid. In contrast, cotransfection of pIR5-CAT with pXG (a plasmid bearing the EHV-1 *Xba*I G restriction fragment in a pAT153 background) resulted in no significant activation of pIR5-CAT (Table 1; Fig. 2, lane 3). However, when pIR5-CAT was cotransfected with pXG in combination with pSVIE (Table 1; Fig. 2, lane 4), CAT activity was induced by approximately 13-fold over basal levels (>4-fold over the level of target gene activation achieved by the IE expression vector alone). To determine which of the three uninterrupted genes (i.e., UL2, UL3, and UL4) within *Xba*I G contributes

TABLE 1. Effect of pSVIE and *Xba*I G clones on pIR5-CAT gene expression

Plasmid(s) transfected with pIR5-CAT	CAT activity (% acetylation)[a]				Fold change[b]
	Expt 1	Expt 2	Expt 3	Mean ± SD	
pUC12	0.36	0.44	0.57	0.46 ± 0.11	
pSVIE	1.25	1.32	1.37	1.31 ± 0.06	2.9
pXG	0.45	0.47	0.51	0.48 ± 0.03	1.0
pXG + pSVIE	4.34	5.06	7.87	5.76 ± 1.87	12.5
pXG44 + pSVIE	5.90	5.98	7.67	6.52 ± 1.00	14.2
pXG45 + pSVIE	6.22	6.69	6.92	6.61 ± 0.36	14.4
pXG46 + pSVIE	1.07	1.08	1.30	1.14 ± 0.13	2.5

[a] Determined as percent conversion of substrate to the acetylated form. Results of three parallel transfection experiments are shown.

[b] Determined by comparison of the average percent acetylation associated with pIR5-CAT in the presence of the indicated effector plasmid(s) versus the average percent acetylation in the presence of pUC12.

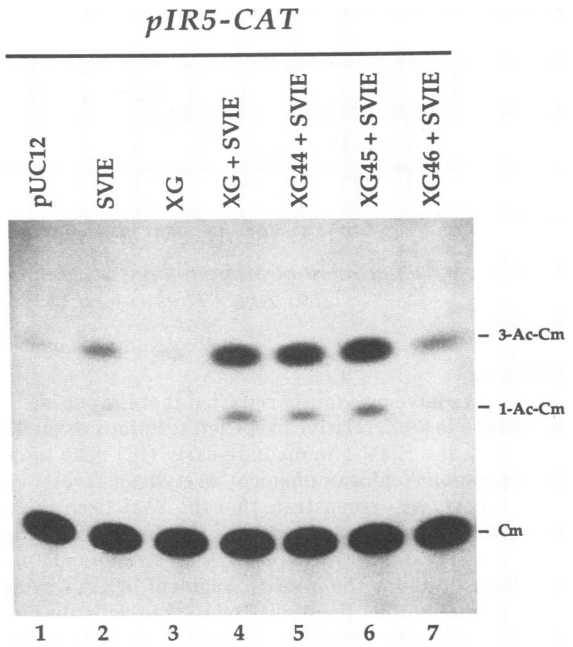

FIG. 2. Representative CAT assay results showing the effects of various plasmids on pIR5-CAT gene expression. L-M cells were transfected with 1.4 pmol of the pIR5-CAT reporter plasmid and 0.3 pmol each of the indicated effector plasmid(s). Plasmid pIR5-CAT contains promoter elements (−1113 to +13) of the EHV-1 IR5 gene (a late gene) linked to CAT coding sequences. Plasmid pSVIE contains the EHV-1 IE open reading frame under the control of the simian virus 40 early promoter. EHV-1 genes contained within *Xba*I G and the *Xba*I G subclones are indicated in Fig. 1. Cells were harvested approximately 62 h posttransfection and assayed for CAT activity as described previously (32). Cm, chloramphenicol; 1-Ac-Cm, 1-acetylchloramphenicol; 3-Ac-Cm, 3-acetylchloramphenicol.

to *Xba*I G activity, a series of *Xba*I G subclones (Fig. 1C) was employed in cotransfection experiments. Plasmid pXG44 contains sequences from nucleotide (nt) 1436 to nt 6510 relative to the left terminus of the EHV-1 genome (Kentucky A strain) and deletes approximately 1.4 kbp from the right end of *Xba*I G but retains the UL2, UL3, and UL4 genes. Cotransfection of pIR5-CAT with pXG44 and pSVIE (Table 1; Fig. 2, lane 5) resulted in an average 14-fold increase in CAT activity compared with that directed by pIR5-CAT in the presence of pUC12. This represents a >4-fold increase in CAT activity compared with that associated with pIR5-CAT in the presence of pSVIE alone. This level of activation is comparable to that obtained by cotransfection of pIR5-CAT with pSVIE and the intact *Xba*I G fragment. Plasmid pXG45 contains sequences from nt 1436 to nt 5249, thus deleting 369 bp from the 5′ end of the UL4 ORF. Cotransfection of pIR5-CAT, pXG45, and pSVIE (Table 1; Fig. 2, lane 6) resulted in an approximately 14-fold induction of CAT activity from pIR5-CAT, a value comparable to that observed for pIR5-CAT in the presence of pXG plus pSVIE or pXG44 plus pSVIE. However, cotransfection of pIR5-CAT with pXG46 (which contains *Xba*I G sequences from nt 1436 to nt 2957 and thus deletes all of UL3, UL4, and the remainder of UL5) and pSVIE yielded an approximately threefold increase in CAT activity which can be attributed to the affects of pSVIE alone (Table 1; Fig. 2, compare lanes 2 and 7). Therefore, pXG46 (which lacks UL3) was unable to contribute to the *trans* activation of pIR5-CAT. These ob-

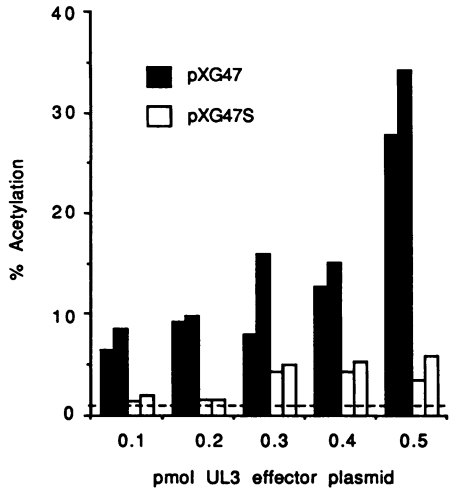

FIG. 3. Effect of increasing amounts of pXG47 and pXG47S on CAT activity in cells transfected with pIR5-CAT and pSVIE. L-M cells were transfected with 1.4 pmol of pIR5-CAT, 0.3 pmol of pSVIE, and the indicated amount of either pXG47 or pXG47S. Plasmid pXG47 bears a single copy of the EHV-1 UL3 gene (Fig. 1C). Plasmid pXG47S is a pXG47 derivative in which a linker (14-mer) containing stop codons in all reading frames was inserted at position 3613 of the UL3 gene. Each datum point shows the results of duplicate transfections which are individually graphed versus percent acetylation. The dashed line represents levels of CAT activity (percent acetylation) associated with pIR5-CAT in the presence of pSVIE alone (i.e., no UL3 effector plasmid).

servations indicate that, of the genes within *Xba*I G, it is UL3 which augments gene expression from pIR5-CAT in the presence of pSVIE.

To confirm that the UL3 gene product is sufficient for cooperative activation of gene expression from pIR5-CAT, the UL3 gene was isolated as a 5.3-kbp *Pst*I-*Sph*I restriction fragment in pUC19 (to generate pXG47) and employed in a dose-response experiment. L-M cells were transfected with 1.4 pmol of the pIR5-CAT reporter plasmid, 0.3 pmol of pSVIE, and increasing amounts (0.0 to 0.5 pmol) of pXG47. As shown in Fig. 3, the addition of as little as 0.1 pmol of pXG47 was sufficient to achieve an approximately fivefold induction of CAT activity over that observed with pIR5-CAT in the presence of pSVIE alone. CAT activity increased with increasing amounts of UL3 effector plasmid, peaking at 0.5 pmol of pXG47 (the largest amount tested). To confirm that it is the coding capacity of UL3 which contributes to induction of pIR5-CAT gene expression, a second version of pXG47 was constructed in which a 14-mer oligonucleotide bearing an amber stop codon in all reading frames was inserted into the unique *Hpa*I site of the UL3 gene at position 3613 to generate pXG47S. The stop codon insertion should result in truncation of the UL3 gene product from 470 to 279 aa and, therefore, the loss of 191 aa from the carboxy terminus of the protein. It has been demonstrated that the carboxy-terminal half of the HSV-1 UL3 homolog (ICP27) is particularly important for protein function (12, 20, 24). Therefore, it was predicted that the UL3 nonsense mutation would severely impair UL3 protein function. This was confirmed by the dose-response experiment diagrammed in Fig. 3. In contrast to the effects of pXG47, expression from pIR5-CAT was not significantly increased by the addition of 0.1 or 0.2 pmol of pXG47S. An approximately 3.5-fold increase in CAT activity was observed with transfection of 0.3 pmol of pXG47S. This level of CAT activity remained approximately the same with the addition of 0.4 and 0.5 pmol of pXG47S. It is possible that this low level of activation is due to titration of a nonspecific repressor of pIR5-CAT expression. Alternatively, the remaining 279 aa of the UL3 gene product may retain some activity which is apparent only at higher molar ratios of effector plasmid to reporter plasmid. However, it is clear from the data that the stop codon insertion within UL3 severely impaired the ability of pXG47S to augment late gene expression. This result indicates that it is the coding capacity of UL3 which is important for *trans* activation of target gene expression from pIR5-CAT.

We have previously reported (32) that while an EHV-1 IE gene expression vector was able to activate a representative EHV-1 early promoter-CAT construct by as much as 60-fold, IE-mediated activation of two representative EHV-1 late promoter-CAT recombinants was relatively poor. It was determined that cotransfection of the EHV-1 *Xba*I G restriction fragment with the IE gene expression vector greatly enhanced CAT activity directed by the late promoter-CAT constructs. Here, we have identified the EHV-1 UL3 gene (ICP27 homolog) as the active component contributed by *Xba*I G in a transient expression assay. This result is consistent with the proposed roles of UL3 and ICP27 as regulatory factors which contribute to the switch from early to late gene expression. HSV-1 mutants which bear defects in the ICP27 gene are impaired in the expression of late infected-cell polypeptides (19, 23, 28). Although EHV-1 strains bearing a mutation within the UL3 gene are not yet available, transient expression assays suggest that UL3 is also important for EHV-1 late gene expression. Transient expression assays employing cloned copies of ICP27 have provided specific, and sometimes conflicting, examples of ICP27-mediated *trans* activation of HSV-1 late promoter-reporter gene constructs. It has been reported that while an ICP27 vector demonstrates little activity when singly transfected with an HSV-1 late promoter-reporter gene chimera containing the VP5 (major capsid protein) promoter linked to CAT, ICP27 can significantly enhance ICP4-ICP0-mediated activation of VP5-CAT (6, 30). In contrast, ICP27 has also been reported to repress *trans* activation of a VP5-CAT construct by ICP4 or ICP0 (20). ICP27 has also been reported to inhibit the *trans* activation of several other HSV-1 promoter-CAT constructs by ICP4 and/or ICP0 (30, 33). Rice and Knipe (22) have reported that ICP27 can activate reporter gene expression directed by an HSV-1 glycoprotein B (gB) promoter-CAT fusion construct and that ICP27 can further enhance ICP4- and/or ICP0-mediated activation of gB-CAT. However, others have observed repression of ICP4- and/or ICP0-mediated *trans* activation of a gB-CAT construct in the presence of ICP27 (30). Rice et al. (24) have reported that ICP27, in the presence of ICP4, can *trans* activate an HSV-1 glycoprotein C (gC) promoter-CAT construct. Similarly, the EHV-1 UL3 gene product significantly enhances reporter gene expression from an EHV-1 gC-CAT construct in the presence of the EHV-1 ICP4 homolog (18). McCarthy et al. (19) have observed that the transcription initiation rate of several HSV genes is decreased in cells infected with an HSV-1 ICP27 null mutant compared with the transcription initiation rate of the same genes in cells infected with wild-type HSV-1. In addition, it has recently been reported that the *trans* effects of ICP27 are related to posttranscriptional processing of target gene mRNAs (29, 31) and that negative effects on gene expression are related to the presence of introns within the target gene while

positive effects are exerted through 3′-end processing of target gene transcripts (29). Although the mechanism by which UL3 contributes to *trans* activation of gene expression has not been determined, increases in steady-state mRNA levels via more efficient processing and/or transport of target gene transcripts are consistent with the effects of UL3 in transient expression assays. A determination of the level at which UL3 functions and an investigation of its protein structure as it relates to function are now underway in our laboratory.

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REFERENCES

1. **Audonnet, J.-C., J. Winslow, G. Allen, and E. Paoletti.** 1990. Equine herpesvirus type 1 unique short fragment encodes glycoproteins with homology to herpes simplex virus type 1 gD, gI and gE. J. Gen. Virol. **71**:2969–2978.
2. **Awan, A. R., Y.-C. Chong, and H. J. Field.** 1990. The pathogenesis of equine herpesvirus type 1 in the mouse: a new model for studying host responses to the infection. J. Gen. Virol. **71**:1131–1140.
3. **Caughman, G. B., A. T. Robertson, W. L. Gray, D. C. Sullivan, and D. J. O'Callaghan.** 1988. Characterization of equine herpesvirus type 1 immediate early proteins. Virology **163**:563–571.
4. **Caughman, G. B., J. Staczek, and D. J. O'Callaghan.** 1985. Equine herpesvirus type 1 infected cell polypeptides: evidence for immediate early/early/late regulation of viral gene expression. Virology **145**:49–61.
5. **Dauenhauer, S. A., R. A. Robinson, and D. J. O'Callaghan.** 1982. Chronic production of defective-interfering particles by hamster embryo cultures of herpesvirus persistently infected and oncogenically transformed cells. J. Gen. Virol. **60**:1–14.
6. **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.
7. **Field, H. J., A. R. Awan, and R. de la Fuente.** 1992. Reinfection and reactivation of equine herpesvirus-1 in the mouse. Arch. Virol. **123**:409–419.
8. **Flowers, C. C., E. M. Eastman, and D. J. O'Callaghan.** 1991. Sequence analysis of a glycoprotein D gene homolog within the unique short segment of the EHV-1 genome. Virology **180**:175–184.
9. **Gray, W. L., R. P. Baumann, A. T. Robertson, G. B. Caughman, D. J. O'Callaghan, and J. Staczek.** 1987. Regulation of equine herpesvirus type 1 gene expression: characterization of immediate early, early, and late transcription. Virology **158**:79–87.
10. **Gray, W. L., R. P. Baumann, A. T. Robertson, D. J. O'Callaghan, and J. Staczek.** 1987. Characterization and mapping of equine herpesvirus type 1 immediate early, early, and late transcripts. Virus Res. **8**:233–244.
11. **Grundy, F. J., R. P. Baumann, and D. J. O'Callaghan.** 1989. DNA sequence and comparative analyses of the equine herpesvirus type 1 immediate early gene. Virology **172**:223–236.
12. **Hardwicke, M. A., P. J. Vaughan, R. E. Sekulovich, R. O'Conner, and R. M. Sandri-Goldin.** 1989. The regions important for the activator and repressor functions of herpes simplex virus type 1 α protein ICP27 map to the C-terminal half of the molecule. J. Virol. **63**:4590–4602.
13. **Harty, R. N., C. F. Colle, F. J. Grundy, and D. J. O'Callaghan.** 1989. Mapping the termini and intron of the spliced immediate-early transcript of equine herpesvirus 1. J. Virol. **63**:5101–5110.
14. **Harty, R. N., C. F. Colle, and D. J. O'Callaghan.** 1991. Equine herpesvirus type 1 gene regulation: characterization of transcription from the IE gene region in a productive infection, p. 319–337. *In* E. K. Wagner (ed.), Herpesvirus transcription and its regulation. CRC Press, Inc., Boca Raton, Fla.
15. **Harty, R. N., and D. J. O'Callaghan.** 1992. Identification and expression of the UL1 gene product of equine herpesvirus 1. Virus Res. **25**:105–116.
16. **Harty, R. N., R. R. Yalamanchili, and D. J. O'Callaghan.** 1991. Transcriptional analysis of the UL1 gene of equine herpesvirus 1: a gene conserved in the genome of defective interfering particles. Virology **183**:830–833.
17. **Holden, V. R., R. R. Yalamanchili, R. N. Harty, and D. J. O'Callaghan.** 1992. Identification and characterization of an equine herpesvirus 1 late gene encoding a potential zinc finger. Virology **188**:704–713.
18. **Matsumura, T., R. H. Smith, and D. J. O'Callaghan.** Unpublished data.
19. **McCarthy, A. M., L. McMahan, and P. A. Schaffer.** 1989. Herpes simplex virus type 1 ICP27 deletion mutants exhibit altered patterns of transcription and are DNA deficient. J. Virol. **63**:18–27.
20. **McMahan, L., and P. A. Schaffer.** 1990. The repressing and enhancing functions of the herpes simplex virus regulatory protein ICP27 map to C-terminal regions and are required to modulate viral gene expression very early in infection. J. Virol. **64**:3471–3485.
21. **O'Callaghan, D. J., G. A. Gentry, and C. C. Randall.** 1983. The equine herpesviruses, p. 215–318. *In* B. Roizman (ed.), The herpesviruses, vol. 2. Comprehensive virology, series 2. Plenum Publishing Corp., New York.
22. **Rice, S. A., and D. M. Knipe.** 1988. Gene-specific transactivation by herpes simplex virus type 1 alpha protein ICP27. J. Virol. **62**:3814–3823.
23. **Rice, S. A., and D. M. Knipe.** 1990. Genetic evidence for two distinct transactivation functions of the herpes simplex virus α protein ICP27. J. Virol. **64**:1704–1715.
24. **Rice, S. A., L. Su, and D. M. Knipe.** 1989. Herpes simplex virus alpha protein ICP27 possesses separable positive and negative regulatory activities. J. Virol. **63**:3399–3407.
25. **Robertson, A. T., G. B. Caughman, W. L. Gray, R. P. Baumann, J. Staczek, and D. J. O'Callaghan.** 1988. Analysis of the *in vitro* translation products of the equine herpesvirus type 1 immediate early mRNA. Virology **166**:451–462.
26. **Robinson, R. A., B. E. Henry, R. G. Duff, and D. J. O'Callaghan.** 1980. Oncogenic transformation by equine herpesviruses (EHV). I. Properties of hamster embryo cells transformed by ultraviolet-irradiated EHV-1. Virology **101**:335–362.
27. **Robinson, R. A., R. B. Vance, and D. J. O'Callaghan.** 1980. Oncogenic transformation by equine herpesviruses. II. Coestablishment of persistent infection and oncogenic transformation of hamster embryo cells by equine herpesvirus type 1 preparations enriched for defective interfering particles. J. Virol. **36**:204–219.
28. **Sacks, W. R., C. C. Greene, D. P. Aschman, and P. A. Schaffer.** 1985. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. J. Virol. **55**:796–805.
29. **Sandri-Goldin, R. M., and G. E. Mendoza.** 1992. A herpesvirus regulatory protein appears to act post-transcriptionally by affecting mRNA processing. Genes Dev. **6**:848–863.
30. **Sekulovich, R. E., K. Leary, and R. M. Sandri-Goldin.** 1988. The herpes simplex virus type 1 α protein ICP27 can act as a *trans*-repressor or a *trans*-activator in combination with ICP4 and ICP0. J. Virol. **62**:4510–4522.
31. **Smith, I. L., M. A. Hardwicke, and R. M. Sandri-Goldin.** 1992. Evidence that the herpes simplex virus immediate early protein ICP27 acts post-transcriptionally during infection to regulate gene expression. Virology **186**:74–86.
32. **Smith, R. H., G. B. Caughman, and D. J. O'Callaghan.** 1992. Characterization of the regulatory functions of the equine herpesvirus 1 immediate-early gene product. J. Virol. **66**:936–945.
33. **Su, L., and D. M. Knipe.** 1989. Herpes simplex virus α protein ICP27 can inhibit or augment viral gene transactivation.

Virology **170:**496–504.
34. **Telford, E. A. R., M. S. Watson, K. McBride, and A. J. Davison.** 1992. The DNA sequence of equine herpesvirus-1. Virology **189:**304–316.
35. **Yalamanchili, R. R., B. Raengsakulrach, and D. J. O'Callaghan.** 1990. Equine herpesvirus 1 sequence near the left terminus codes for two open reading frames. Virus Res. **18:**109–116.
36. **Zhao, Y., V. R. Holden, R. N. Harty, and D. J. O'Callaghan.** 1992. Identification and transcriptional analyses of the UL3 and UL4 genes of equine herpesvirus 1, homologs of the ICP27 and glycoprotein K genes of herpes simplex virus. J. Virol. **66:**5363–5372.