JOURNAL OF VIROLOGY, Feb. 2004, p. 1063–1068 Vol. 78, No. 3
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.3.1063–1068.2004

MINIREVIEW

Herpes Simplex Virus Virion Host Shutoff Protein: Immune Evasion Mediated by a Viral RNase?

James R. Smiley*

Department of Medical Microbiology & Immunology, University of Alberta, Edmonton, Alberta, Canada T6G 2S2

Herpes simplex virus type 1 (HSV-1) is the prototypical member of the *Herpesviridae*, a large family of enveloped DNA viruses that infect diverse metazoans. It is also the defining example of the *Alphaherpesvirinae*, the neurotropic subfamily of herpesviruses. Like all herpesviruses, HSV displays both lytic and latent modes of interaction with its natural human host. Primary infection of epithelial cells produces the lytic response—virus replication followed by cell death. Progeny virus particles then infect adjacent sensory neurons, establishing a lifelong latent interaction. The latent viral genome is maintained in an extrachromosomal state in which only a restricted portion of the genome is transcribed. Latent genomes occasionally reactivate into the lytic cycle, producing a limited amount of progeny virus that gives rise to secondary infections of the epithelial sites enervated by the latently infected neurons.

HSV executes a complex genetic program during lytic infection (reviewed in reference 47). Expression of most cellular genes is strongly suppressed, and three temporal classes of viral genes are sequentially activated in a regulatory cascade. Five viral immediate-early (IE) genes are expressed first, and four of these (ICP0, ICP4, ICP22, and ICP27) encode regulatory proteins that stimulate expression of the viral early (E) and late (L) genes. The E genes are activated next, giving rise to proteins required for replication of the viral genome. Viral DNA replication then ensues, augmenting IE-dependent expression of the L genes that encode the structural components of the virion.

HSV differs from many other nuclear DNA viruses in that some of its key regulatory polypeptides are delivered into the host cell by the infecting virus particle. These virion regulators are located in the viral tegument—the space between the envelope and the nucleocapsid—and as such are injected into the newly infected cell immediately upon fusion of the viral envelope with the host cell plasma membrane. These proteins are therefore strategically poised to influence the very earliest events in the viral replication cycle. In the best-known case, the abundant tegument protein VP16 activates transcription of the viral IE genes, thereby contributing to the initial launch of the lytic program of gene expression (reviewed in reference 17). The tegument also contains *vhs*, the virion host shutoff protein encoded by HSV gene UL41. *vhs* is an mRNA-specific RNase that triggers rapid shutoff of host cell protein synthesis, disruption of preexisting polyribosomes, and degradation of host mRNAs in the absence of de novo viral gene expression (reviewed in reference 55). Here I summarize our present understanding of the mechanism of *vhs* action and discuss recent studies that point to intriguing roles in viral pathogenesis and immune evasion. Space limitations preclude an exhaustive review of the earlier literature; I therefore seek the indulgence of my colleagues and refer the interested reader to a recent review (55) and the introductory sections of two recent articles (9, 10) for more details. Unless otherwise stated below, *vhs* refers to the UL41 gene product of HSV-1.

GENERAL FEATURES OF HSV-INDUCED HOST SHUTOFF: CONTEXT OF *vhs* ACTION

HSV infection leads to essentially complete suppression of cellular protein synthesis. This global shutoff stems from at least two distinct inhibitory pathways. First, the levels of most host mRNAs undergo a precipitous decline (12, 26), curtailing synthesis of the corresponding proteins. *vhs* contributes to this decline by globally increasing the rate of mRNA degradation in the cytoplasm (26, 63). The effect of *vhs* is magnified by virus-induced suppression of host mRNA synthesis, mediated through repression of primary transcription (59) and pre-mRNA splicing (16). As described below, the multifunctional IE protein ICP27 plays a particularly prominent role in the inhibition of host mRNA biogenesis at both of these levels. ICP27 thus collaborates with *vhs* to reduce the abundance of host mRNA during infection (15, 57). Second, HSV alters the function of the host translational apparatus, such that translation of the residual portion of many of the down-regulated cellular mRNAs is strongly impaired (14, 27). This effect is probably due to impaired initiation, as a significant fraction of the residual mRNAs is found in 48S translational preinitiation complexes (27). Little is known of the mechanisms underlying this translational control. However, *vhs* is apparently not involved (27) and this topic will not be considered further in this minireview.

RNA polymerase II (RNAP II) transcription of many (but not all [2, 21]) cellular genes is strongly suppressed (59), while RNAP II transcription of the viral genome is activated to high levels by the viral IE proteins. Early studies indicated that this major shift in the activity of the cellular transcription machinery does not involve sequence-specific discrimination between

* Mailing address: Department of Medical Microbiology & Immunology, 632 Heritage Medical Research Centre, University of Alberta, Edmonton, Alberta, Canada, T6G 2S2. Phone: (780) 492-4070. Fax: (780) 492-9828. E-mail: jim.smiley@ualberta.ca.

cellular and viral RNAP II promoters (reviewed in reference 56). The shift is, however, mirrored by a striking relocalization of RNAP II and associated general transcription factors from cellular chromatin to viral replication compartments (45), the sites of viral genome replication and transcription in the nucleus. The molecular basis for the impaired transcription of host genes remains unclear. It precedes global relocalization of RNAP II and is independent of viral DNA replication, implying that it does not stem from simple competition between viral and cellular promoters (59). Viral IE gene expression is required, but no single IE protein is essential, suggesting significant functional redundancy among the IE proteins in mediating the repressive effects. However, ICP27 appears to make a stronger contribution to transcriptional repression of host genes than the other IE proteins (59).

HSV induces several changes in the properties of the RNAP II holoenzyme complex that may contribute to the global shift in transcription from cellular to viral genes. First, the phosphorylation state of the carboxy-terminal domain of the largest subunit of RNAP II is altered (45). The hyperphosphorylated form (RNAP II0) characteristic of transcriptional elongation in uninfected cells disappears and is replaced by a virus-specific, intermediately phosphorylated form termed RNAP II_i. The depletion of RNAP II0 and accumulation of RNAP II_i can be partially uncoupled by mutation, for example, by inactivating IE protein ICP22 or the UL13 protein kinase (32, 44). Under these conditions II0 is depleted but little II_i is formed, leading to an accumulation of the hypophosphorylated RNAP IIA form characteristic of unengaged RNAP II. Studies of these and other mutants suggest a strong correlation between the loss of II0 and repression of cellular transcription (59). Second, infection displaces the essential general transcription factor TFIIE from the RNAP II holoenzyme (19). Third, several HSV proteins, including ICP4, ICP27, and ICP8, have been reported to associate with RNAP II in either the holoenzyme complex (71) or a smaller complex found in infected cells (19). Jenkins and Spencer (19) suggest that these alterations may act in combination to selectively impair transcription of chromatin-associated templates, thus repressing cellular genes and favoring transcription of the nonchromatinized viral genome. This is an intriguing model, because it proposes that differences in chromatin structure underlie the global switch from cellular to viral transcription during infection. As such, it may help explain two otherwise puzzling findings that were made well over a decade ago: (i) HSV E and L promoters are composed of binding sites for cellular transcription factors and lack virus-specific sequences required for activation by viral IE proteins (reviewed in reference 7), and (ii) a virally transduced copy of the cellular β-globin gene is activated by viral IE proteins while the corresponding endogenous chromosomal gene is transcriptionally repressed during infection of erythroid cells with an HSV recombinant (54).

HSV infection also impairs mRNA splicing, and ICP27 is both necessary and sufficient for this effect (reviewed in references 51 and 53). The majority of cellular genes contain introns, while most HSV genes do not (only four of the ca. 84 viral genes contain introns, and three of these are IE genes). Thus, repression of splicing is predicted to inhibit cellular gene expression without affecting the expression of most HSV genes. ICP27 inhibits splicing in vitro, upstream of the first catalytic step, implying a direct mode of action on spliceosome assembly or function (1, 31, 53). Indeed, recent studies demonstrate that ICP27 directly binds splicing factors, including SAP145 and several SR proteins (1, 53). It also binds the SR kinase SRK1 and alters its activity, leading to accumulation of hypophosphorylated forms of SR proteins (53). Sciabica et al. (53) suggest that aberrant phosphorylation of SR proteins is the critical ICP27-induced event that prevents spliceosome assembly and thus blocks splicing.

Genetic studies demonstrate that ICP27 is required for efficient shutoff of host protein synthesis during HSV infection (35, 48) and contributes (along with *vhs*) to the loss of host mRNAs (15, 57). These two effects are often equated and ascribed to the splicing inhibition function of ICP27. However, while inhibition of splicing undoubtedly contributes to mRNA loss and inhibition of protein synthesis, it is important to stress that ICP27 is a multifunctional protein that also represses transcription of host genes (59); in addition, recent data suggest that ICP27 is also required for translational repression of at least some cellular mRNAs (K. S. Ellison, R. A. Maranchuk, and J. R. Smiley, unpublished data). The relative importance of the various activities of ICP27 to host shutoff remains to be determined.

MECHANISM OF *vhs* ACTION

vhs triggers global mRNA destabilization in infected cells, an effect that is generally assumed to fully account for its role in host shutoff (26, 41, 43). Several lines of evidence combine to provide a compelling case that *vhs* is either an RNase or a required subunit of an RNase that also contains one or more cellular subunits. First, HSV *vhs* and its orthologues in the other alphaherpesviruses display significant amino acid sequence similarity to a family of cellular nucleases that are involved in DNA replication and repair (4, 8, 9). This similarity can be readily visualized following three iterations of the PSI-Blast program with the default parameters at www.ncbi.nih.gov/blast (accession number for *vhs*: NP_044643). One of the most highly related nonviral proteins is FEN-1, an endo/exonuclease that helps remove the RNA primers from Okazaki fragments during DNA replication in eukaryotes (30). The sequence similarity to cellular nucleases appears to be critical for *vhs* function, as mutations that alter conserved residues essential for the catalytic activity of cellular homologues eliminate the ability of *vhs* to inhibit reporter gene expression in a transient-cotransfection assay (9). Second, extracts of HSV-infected cells and partially purified virions contain an RNase activity that is eliminated by *vhs* mutations and inhibited by anti-*vhs* antibodies (24, 58, 70). In addition, *vhs* induces RNA degradation via endonucleolyic cleavage when it is expressed as the only HSV protein in a rabbit reticulocyte lysate (RRL) in vitro translation system (5, 70) or in the budding yeast *Saccharomyces cerevisiae* (R. C. Doepker and J. R. Smiley, unpublished data). Third, a partially purified complex of *vhs* and the mammalian translation initiation factor eIF4H isolated from *Escherichia coli* displays RNase activity that is eliminated by mutations in the nuclease domain of *vhs* (9). Unfortunately, *vhs* has yet to be purified to homogeneity in a soluble form, and so it is not clear if *vhs* has significant nuclease activity in the absence of cellular factors such as eIF4H.

The *vhs*-dependent RNase exhibits a high degree of functional specificity in vivo, as it apparently targets only mRNAs and spares other cytoplasmic RNA species (24, 26, 40, 70). Both cellular and viral mRNAs are affected (26, 41), with no definitive evidence to date for selective targeting of particular mRNA species. The resulting global reduction in mRNA stability in infected cells is well suited to the requirements of a rapidly replicating virus such as HSV, as it facilitates quick changes in mRNA levels in response to altered synthetic rates. For most cellular genes, the net effect is a dramatic decline in mRNA levels leading to host shutoff. The reduced stability of viral mRNAs also helps to sharpen the transitions between the successive phases of viral protein synthesis, by tight changes in the transcription rates of individual viral genes to altered mRNA levels (26, 41).

How does the *vhs*-dependent RNase selectively target mRNAs as opposed to other cellular RNAs? The possibility that it directly recognizes the 5′ cap or 3′ poly(A) tail is apparently excluded by observations indicating that neither of these RNA modifications is required for substrate recognition in in vitro reactions (5, 22, 70). However, some evidence suggests that *vhs* is preferentially targeted to regions of translation initiation on mRNAs. Thus, the 5′ end of HSV thymidine kinase mRNA is degraded before the 3′ end in vivo (22), and the sites of initial cleavage of signal recognition particle α mRNA are nonrandomly clustered over the 5′ region of this transcript in the RRL in vitro assay system (5). In addition, the internal ribosome entry sites (IRES) of encephalomyocarditis virus and poliovirus strongly target the initial sites of *vhs*-induced cleavage to the immediate 3′-flanking sequences (6). Taken together, these data raise the possibility that *vhs* is targeted to mRNAs through interactions with one or more components of the cellular translation initiation machinery. Consistent with this hypothesis, Feng et al. have shown that *vhs* directly binds the cellular translation initiation factor eIF4H (10). eIF4H displays amino acid sequence and functional similarity to eIF4B: both proteins stimulate the helicase and ATPase activity of eIF4A (a component of the cap-binding complex eIF4F) (46) and act along with other eIF4 factors at an early stage in translation initiation. Feng et al. suggest that the *vhs*-eIF4H interaction serves to target *vhs* to sites of translation initiation on actively translated mRNAs (e.g., the 5′ end of capped mRNAs). This elegant model has yet to be directly tested. However, it is worth noting that Taddeo et al. (65) have suggested that the cellular stress-inducible IEX-1 mRNA is degraded from the 3′ (rather than 5′) end in a *vhs*-dependent fashion, raising the possibility that *vhs* is not invariably targeted to sites of translational initiation on susceptible mRNAs.

The interaction of *vhs* with eIF4H suggests that the activity and/or mRNA targeting properties of *vhs* may be regulated by cellular factors. Consistent with this possibility, Lu et al. (33) found that one or more factors present in RRLs strongly stimulate the nuclease activity of *vhs* expressed in the budding yeast *S. cerevisiae*. By using this system, we have recently found that eIF4H and eIF4B are each capable of stimulating the in vitro nuclease activity of *vhs* (Doepker and Smiley, unpublished). However, neither factor is able to reconstitute IRES-directed targeting, implying that additional mammalian factors are required.

BIOLOGICAL ROLES OF *vhs*

vhs homologues are present in all of the alpha- (neurotropic) herpesvirus genomes that have been sequenced to date yet are absent from the beta- and gammaherpesviruses. Thus, *vhs* likely plays a fundamental and conserved role in the biology of alphaherpesvirus infections. It is therefore interesting and perhaps surprising that *vhs*-null mutations have only a relatively modest effect on virus replication in tissue culture (5- to 10-fold reduction [42, 43, 54]). However, such mutations severely impair virus replication and pathogenesis in mouse models of HSV infections and preclude establishment and possibly reactivation from latency (29, 60–62). The magnitude of the peripheral replication impairment in mice is impressive: *vhs* mutants are more defective than mutants lacking the IE protein ICP0 (29), in striking contrast to the situation in cell culture where ICP0 mutations have a much greater effect on virus replication. The remarkable difference between the phenotypes of *vhs* mutants in tissue culture and animal models indicates that *vhs* is arguably a bona fide HSV virulence factor. The basis for the severe attenuation of *vhs* mutants remains to be completely defined, but accumulating evidence suggests that it may stem, at least in part, from defects in viral evasion of host immunity.

vhs-induced mRNA decay contributes (along with IE protein ICP47) to the loss of major histocompatibility complex (MHC) class I from the cell surface (18, 23, 66) and thus helps render infected cells resistant to lysis by cytotoxic T lymphocyes (at least during infection with HSV-2 and bovine herpesvirus 1). Similarly, *vhs* reportedly contributes to reduced levels of MHC class II (67), an effect that is predicted to impair antigen presentation and blunt both cellular and humoral responses. In addition, *vhs* suppresses the production of proinflammatory chemokines and cytokines, including interleukin 1β (IL-1β), IL-8, and MIP-1α in infected U937 cells and IL-8 in HEL cells (64). It may also contribute to the interferon-resistant phenotype of HSV in some (but not all [37]) cell types in tissue culture (64). Perhaps most strikingly, *vhs* has been recently shown to functionally inactivate human monocyte-derived dendritic cells (50). Previous work had demonstrated that HSV-infected dendritic cells are functionally impaired in their activation properties and ability to stimulate T cells (25, 49). Samady et al. (50) have now shown that these effects require *vhs* and occur in the absence of appreciable viral gene expression. Thus, in the presence of *vhs*, infected dendritic cells fail to upregulate activation markers in response to HSV infection or exposure to lipopolysaccharide. In contrast, the cells were strongly activated following infection with a *vhs*-null mutant. These findings are quite remarkable, as the levels of *vhs* delivered by the infecting virus particle are not overtly toxic in other cell types (20) and only partially inhibit global cellular protein synthesis (43). The ability of *vhs* to quench the activation of at least some antigen-presenting dendritic cell subtypes provides additional evidence that it may play a role in dampening both arms of the adaptive immune response to HSV infection. However, it is important to note that *vhs* does not globally block the activation of all dendritic cell subtypes. Lund et al. (34) have recently shown that wild-type HSV-1 and HSV-2 virions (which contain functional *vhs*) efficiently trigger mouse bone marrow-derived plasmacytoid dentritic cells to

produce alpha interferon, by signaling through Toll-like receptor 9 (TLR9). In this case the virion is probably delivered by endocytosis to an intracellular acidic vesicular compartment where it is degraded, releasing the viral DNA that activates TLR9 (34). Presumably, the *vhs* present in these doomed endocytosed virions does not enter the cytoplasm, perhaps accounting for its inability to block activation of this dendritic cell subtype.

Taken collectively, the results summarized above raise the possibility that *vhs* may help HSV to evade host innate and acquired immune responses. This leads to several intriguing and interrelated questions. First, does *vhs* significantly impair antiviral immunity during infection in animal models? Second, if so, to what extent does impaired immune evasion contribute to the severe attenuation of *vhs*-null mutants in vivo? Third, do the various potential immune evasion attributes of *vhs* described above stem from its RNase activity, and if so, does *vhs* selectively target certain cellular mRNAs over others?

Regarding the first question, *vhs*-null mutants have been shown to serve as highly effective protective vaccines in a mouse model and are even capable of reducing reactivation when administered prophylactically to mice already latently infected with wild-type virus (68, 69). But are *vhs* mutants more effective than a matched vhs^+ control, as would be predicted if *vhs* does indeed function to suppress host immunity? The extreme virulence of wild-type HSV precludes a simple comparison between *vhs*-null virus and wild-type virus in mouse models. Geiss et al. (13) have adroitly addressed these issues by comparing the vaccine properties of matched vhs^+ and *vhs*-null versions of a replication-incompetent virus bearing a deletion in the essential DNA replication protein ICP8. The results were clear: the vhs^- virus was significantly more effective as an immunogen than the vhs^+ strain, and both cellular and humoral responses were augmented. Remarkably, the vhs^-/$ICP8^-$ virus was as effective as a replication-competent vhs^-/$ICP8^+$ virus. These data provide a strong indication that *vhs* does in fact help to suppress the acquired immune response to HSV, at least in mice.

Considering the second question, *vhs* mutants display severely reduced levels of virus replication relative to wild-type virus even at the earliest time points analyzed in mouse models (60, 62). Thus, if the attenuation of *vhs* mutants stems at least in part from impaired evasion of host immunity, the innate immune response likely plays a particularly prominent role. Consistent with this view, the attenuated phenotype of HSV-1 *vhs* mutants is to some degree alleviated in knockout mice lacking the alpha/beta interferon receptor (29). However, the effect is not as striking as would have been expected if the interferon response were the main target of *vhs*. In contrast, recent studies by Murphy et al. (38) demonstrate that the virulence of HSV-2 *vhs* mutants is almost fully restored in these mutant mice. The effect is specific to *vhs*, as the host mutation did not restore virulence to an HSV-2 mutant lacking thymidine kinase. These data imply that HSV-1 *vhs* and HSV-2 *vhs* make functionally distinct contributions to disarming host defense mechanisms. They also indicate that the attenuated phenotype of HSV-2 *vhs* mutants stems largely from their inability to counteract the host interferon system, as opposed to any defects in viral gene expression, DNA replication, or virion assembly at the level of the individual infected cell. Along similar lines, elegant and incisive experiments by Suzutani et al. (64) have provided strong evidence for the involvement of the innate immune response in the attenuation of HSV-1 *vhs* mutants in a mouse encephalitis model. They showed that, while a *vhs*-null mutant is essentially avirulent following intracranial inoculation, virulence is almost completely restored following low-dose whole-body gamma irradiation. The effect was specific, in that the virulence of an HSV-1 thymidine kinase mutant was not altered. Remarkably, irradiation of the site of infection (the head) had no such alleviatory effect. These results are very difficult to reconcile with the hypothesis that the severe attenuation of HSV-1 *vhs* mutants stems from defects in virus production at the level of the individual infected cell. Rather, the authors suggested that replication of the *vhs* mutant is suppressed in vivo by radiosensitive immune effector cells (such as neutrophils or lymphocytes) that are recruited from the periphery to the site of infection. The implication is that, in the absence of *vhs*, infected cells are induced to secrete one or more chemokines or cytokines that recruit immune effector cells (i.e., *vhs* serves to block this host response to HSV infection). Consistent with this hypothesis, as noted above, the *vhs* mutant used in this study was shown to induce the synthesis of IL-8, IL-1β, and MIP1-α following infection of the U937 monocytic cell line, while wild-type virus had no such effect (64). Taken collectively, the data obtained from in vivo studies of *vhs*-null mutants of HSV-1 and HSV-2 indicate that their attenuated phenotype stems at least in part from their inability to disarm elements of the innate immune response. However, the data do not exclude the possibility that cell-autonomous defects in virus replication also contribute to attenuation, for example, by severely reducing virus replication in specific cell types.

The ability of *vhs* to quench the induction of cytokine and chemokine expression in U937 cells and suppress activation of monocyte-derived dendritic cells is very intriguing, for at least two reasons. First, both responses are actively induced by HSV infection in the absence of *vhs*, implying that they represent cellular antiviral responses. Indeed, although the mechanisms of host gene induction in these situations have yet to be established, the responses are reminiscent of those elicited by other viruses by signaling through Toll-like receptors (see, for example, reference 3). Second, these responses seem to be almost completely suppressed by *vhs*. The reported magnitude of the inhibitory effects is quite surprising, because, as noted above, *vhs* does not generally induce a complete blockade of cellular protein synthesis and is not overtly toxic. In addition, some previous studies of HSV-induced antiviral responses indicate that *vhs* does not serve a globally countermanding viral immune evasion function. For example, HSV virions induce ca. 30 cellular interferon-stimulated genes (ISGs) in the absence of viral gene expression in fibroblasts (36, 39). The virions used in those studies contained fully active *vhs*, indicating that *vhs* does not eliminate ISG induction. Unpublished data confirm that *vhs* does not greatly affect the level of induction of ISG 56K mRNA by HSV virions (W.-L. Hsu and J. R. Smiley, unpublished data). One possibility is that the apparent differences in susceptibility between various host mRNAs stem from differential effects at the levels of mRNA synthesis, processing, or transport, rather than any specificity of *vhs* action. For example, mRNA transcribed from genes that are strongly in-

duced by infection may accumulate at the same time that those derived from less robustly transcribed genes are declining in abundance. Alternatively, it is possible that *vhs* exhibits some measure of specificity, strongly suppressing the expression of a small subset of cellular genes, including some (but not all) of those involved in host antiviral responses, while having substantially less effect on the majority of other cellular genes. If so, uncovering the basis for this selectivity will be of considerable interest. In this case, it would be especially important to determine if the suppression of any preferred targets of *vhs* action is mediated by the RNase activity of *vhs* or instead stems from another previously unknown function of this protein. In this context, it is interesting that a small fraction of *vhs* associates with lipid rafts in cellular membranes (28), perhaps suggesting a role in cellular signal transduction pathways.

The possibility that *vhs* selectively impairs the expression of a small subset of key cellular genes might help explain the otherwise puzzlingly broad range of generalized host shutoff activity displayed by the various *vhs* homologues of alphaherpesviruses. Thus, HSV-2 *vhs* is approximately 50-fold more active than that of HSV-1 (see, for example, reference 8), while pseudorabies virus *vhs* and varicella-zoster virus *vhs* are substantially less active (5, 52). In addition, although the *vhs* homologue of equine herpesvirus 1 (EHV-1) displays shutoff activity in a transient-cotransfection assay and is packaged into virions, EHV-1 virions do not detectably inhibit general cellular protein synthesis (11). These observations are difficult to reconcile with the idea that global host shutoff is the biologically relevant conserved function of *vhs*. Therefore, if HSV-1 *vhs* proves to display target selectivity, it will be very interesting to determine if this is also the case for the other *vhs* homologues.

In addition, or alternatively, the highly varied host shutoff activities of the various *vhs* homologues may point to the existence of another function of *vhs* that accounts for its strong conservation in the genomes of alphaherpesviruses. In this context it is interesting that the cellular nucleases such as FEN-1 that display the greatest amino acid sequence similarity to *vhs* are involved in DNA replication and repair rather than mRNA metabolism. For example, FEN-1 helps remove the RNA primers from Okazaki fragments (30). Although HSV and other herpesviruses encode their own DNA replication machinery (which presumably requires a FEN-1-like activity), there is presently no evidence that HSV *vhs* participates in viral DNA replication. However, this possibility cannot be discounted, especially in noncycling cells in vivo. Indeed, a varicella-zoster virus *vhs*-null mutant displays a severe temperature-sensitive defect in viral DNA replication in tissue culture (52).

Further studies will reveal whether or not the foregoing speculations on *vhs* function have any merit.

ACKNOWLEDGMENTS

Research in my laboratory is supported by operating grants from the Canadian Institutes of Health Research.

I thank Michele Barry, Deborah Burshtyn, Larry Guilbert, Hanne Ostergaard, and Luis Schag for helpful discussions.

REFERENCES

1. **Bryant, H. E., S. E. Wadd, A. I. Lamond, S. J. Silverstein, and J. B. Clements.** 2001. Herpes simplex virus IE63 (ICP27) protein interacts with spliceosome-associated protein 145 and inhibits splicing prior to the first catalytic step. J. Virol. **75:**4376–4385.
2. **Cheung, P., B. Panning, and J. R. Smiley.** 1997. Herpes simplex virus immediate-early proteins ICP0 and ICP4 activate the endogenous human α-globin gene in nonerythroid cells. J. Virol. **71:**1748–1793.
3. **Compton, T., E. A. Kurt-Jones, K. W. Boehme, J. Belko, E. Latz, D. T. Golenbock, and R. W. Finberg.** 2003. Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2. J. Virol. **77:**4588–4596.
4. **Doherty, A. J., L. C. Serpell, and C. P. Ponting.** 1996. The helix-hairpin-helix DNA-binding motif: a structural basis for non-sequence-specific recognition of DNA. Nucleic Acids Res. **24:**2488–2497.
5. **Elgadi, M. M., C. E. Hayes, and J. R. Smiley.** 1999. The herpes simplex virus Vhs protein induces endoribonucleolytic cleavage of target RNAs in cell extracts. J. Virol. **73:**7153–7164.
6. **Elgadi, M. M., and J. R. Smiley.** 1999. Picornavirus internal ribosome entry site elements target RNA cleavage events induced by the herpes simplex virus virion host shutoff protein. J. Virol. **73:**9222–9231.
7. **Everett, R. D.** 1987. The regulation of transcription of viral and cellular genes by herpesvirus immediate-early gene products. Anticancer Res. **7:**589–604.
8. **Everly, D. N., Jr., and G. S. Read.** 1997. Mutational analysis of the virion host shutoff gene (UL41) of herpes simplex virus (HSV): characterization of HSV type 1 (HSV-1)/HSV-2 chimeras. J. Virol. **71:**7157–7166.
9. **Everly, D. N. J., P. Feng, I. S. Mian, and G. S. Read.** 2002. mRNA degradation by the virion host shutoff (Vhs) protein of herpes simplex virus: genetic and biochemical evidence that Vhs is a nuclease. J. Virol. **76:**8560–8571.
10. **Feng, P., D. N. J. Everly, and G. S. Read.** 2001. mRNA decay during herpesvirus infections: interaction between a putative viral nuclease and a cellular translation factor. J. Virol. **75:**10272–10280.
11. **Feng, X., Y. G. Thompson, J. B. Lewis, and G. B. Caughman.** 1996. Expression and function of the equine herpesvirus 1 virion-associated host shutoff homolog. J. Virol. **70:**8710–8718.
12. **Fenwick, M. L., and M. M. McMenamin.** 1984. Early virion-associated suppression of cellular protein synthesis by herpes simplex virus is accompanied by inactivation of mRNA. J. Gen. Virol. **65:**1225–1228.
13. **Geiss, B. J., T. J. Smith, D. A. Leib, and L. A. Morrison.** 2000. Disruption of virion host shutoff activity improves the immunogenicity and protective capacity of a replication-incompetent herpes simplex virus type 1 vaccine strain. J. Virol. **74:**11137–11144.
14. **Greco, A., A. M. Laurent, and J. J. Madjar.** 1997. Repression of β-actin synthesis and persistence of ribosomal protein synthesis after infection of HeLa cells by herpes simplex virus type 1 are under translational control. Mol. Gen. Genet. **256:**320–327.
15. **Hardwicke, M. A., and R. M. Sandri-Goldin.** 1994. The herpes simplex virus regulatory protein ICP27 contributes to the decrease in cellular mRNA levels during infection. J. Virol. **68:**4797–4810.
16. **Hardy, W. R., and R. M. Sandri-Goldin.** 1994. Herpes simplex virus inhibits host cell splicing, and regulatory protein ICP27 is required for this effect. J. Virol. **68:**7790–7799.
17. **Herr, W.** 1998. The herpes simplex virus VP16-induced complex: mechanisms of combinatorial transcriptional regulation. Cold Spring Harbor Symp. Quant. Biol. **63:**599–607.
18. **Hinkley, S., A. P. Ambagala, C. J. Jones, and S. Srikumaran.** 2000. A vhs-like activity of bovine herpesvirus-1. Arch. Virol. **145:**2027–2046.
19. **Jenkins, H. L., and C. A. Spencer.** 2001. RNA polymerase II holoenzyme modifications accompany transcription reprogramming in herpes simplex virus type 1-infected cells. J. Virol. **75:**9872–9884.
20. **Johnson, P. A., M. J. Wang, and T. Friedmann.** 1994. Improved cell survival by the reduction of immediate-early gene expression in replication-defective mutants of herpes simplex virus type 1 but not by mutation of the virion host shutoff function. J. Virol. **68:**6347–6362.
21. **Kanangat, S., J. S. Babu, D. M. Knipe, and B. T. Rouse.** 1996. HSV-1-mediated modulation of cytokine gene expression in a permissive cell line: selective upregulation of IL-6 gene expression. Virology **219:**295–300.
22. **Karr, B. M., and G. S. Read.** 1999. The virion host shutoff function of herpes simplex virus degrades the 5′ end of a target mRNA before the 3′ end. Virology **264:**195–204.
23. **Koppers-Lalic, D., F. A. Rijsewijk, S. B. Verschuren, J. A. van Gaans-Van den Brink, A. Neisig, M. E. Ressing, J. Neefjes, and E. J. Wiertz.** 2001. The UL41-encoded virion host shutoff (vhs) protein and vhs-independent mechanisms are responsible for down-regulation of MHC class I molecules by bovine herpesvirus 1. J. Gen. Virol. **82:**2071–2081.
24. **Krikorian, C. R., and G. S. Read.** 1991. In vitro mRNA degradation system to study the virion host shutoff function of herpes simplex virus. J. Virol. **65:**112–122.
25. **Kruse, M., O. Rosorius, F. Kratzer, G. Stelz, C. Kuhnt, G. Schuler, J. Hauber, and A. Steinkasserer.** 2000. Mature dendritic cells infected with herpes simplex virus type 1 exhibit inhibited T-cell stimulatory capacity. J. Virol. **74:**7127–7136.
26. **Kwong, A. D., and N. Frenkel.** 1987. Herpes simplex virus-infected cells

contain a function(s) that destabilizes both host and viral mRNAs. Proc. Natl. Acad. Sci. USA **84:**1926–1930.
27. **Laurent, A. M., J. J. Madjar, and A. Greco.** 1998. Translational control of viral and host protein synthesis during the course of herpes simplex virus type 1 infection: evidence that initiation of translation is the limiting step. J. Gen. Virol. **79:**2765–2775.
28. **Lee, G. E., G. A. Church, and D. W. Wilson.** 2003. A subpopulation of tegument protein vhs localizes to detergent-insoluble lipid rafts in herpes simplex virus-infected cells. J. Virol. **77:**2038–2045.
29. **Leib, D. A., T. E. Harrison, K. M. Laslo, M. A. Machalek, N. J. Moorman, and H. W. Virgin.** 1999. Interferons regulate the phenotypes of wild-type and mutant herpes simplex viruses in vivo. J. Exp. Med. **189:**663–672.
30. **Lieber, M. R.** 1997. The FEN-1 family of structure-specific nucleases in eukaryotic DNA replication, recombination and repair. Bioessays **19:**233–240.
31. **Lindberg, A., and J. P. Kreivi.** 2002. Splicing inhibition at the level of spliceosome assembly in the presence of herpes simplex virus protein ICP27. Virology **294:**189–198.
32. **Long, M. C., V. Leong, P. A. Schaffer, C. A. Spencer, and S. A. Rice.** 1999. ICP22 and the UL13 protein kinase are both required for herpes simplex virus-induced modification of the large subunit of RNA polymerase II. J. Virol. **73:**5593–5604.
33. **Lu, P., F. E. Jones, H. A. Saffran, and J. R. Smiley.** 2001. The herpes simplex virus virion host shutoff protein requires a mammalian factor for efficient in vitro endoribonuclease activity. J. Virol. **75:**1172–1185.
34. **Lund, J., A. Sato, S. Akira, R. Medzhitov, and A. Iwasaki.** 2003. Toll-like receptor 9-mediated recogntion of herpes simplex virus-2 by plasmacytoid dendritic cells. J. Exp. Med. **198:**513–520.
35. **McCarthy, A. M., L. McMahan, and P. A. Schaffer.** 1989. Herpes simplex virus type 1 ICP27 deletion mutant exhibits altered patterns of transcription and are DNA deficient. J. Virol. **63:**18–27.
36. **Mossman, K. L., P. F. Macgregor, J. J. Rozmus, A. B. Goryachev, A. M. Edwards, and J. R. Smiley.** 2001. Herpes simplex virus triggers and then disarms a host antiviral response. J. Virol. **75:**750–758.
37. **Mossman, K. L., H. A. Saffran, and J. R. Smiley.** 2000. Herpes simplex virus ICP0 mutants are hypersensitive to interferon. J. Virol. **74:**2052–2056.
38. **Murphy, J. A., R. J. Duesrt, T. J. Smith, and L. A. Morrison.** 2003. Herpes simplex virus type 2 virion host shutoff protein regulates alpha/beta interferon but not adaptive immune responses during primary infection in vivo. J. Virol. **77:**9337–9345.
39. **Nicholl, M. J., L. H. Robinson, and C. M. Preston.** 2000. Activation of cellular interferon-responsive genes after infection of human cells with herpes simplex virus type 1. J. Gen. Virol. **81:**2215–2218.
40. **Oroskar, A. A., and G. S. Read.** 1989. Control of mRNA stability by the virion host shutoff function of herpes simplex virus. J. Virol. **63:**1897–1906.
41. **Oroskar, A. A., and G. S. Read.** 1987. A mutant of herpes simplex virus type 1 exhibits increased stability of immediate-early (*alpha*) mRNAs. J. Virol. **61:**604–606.
42. **Read, G. S., B. M. Bradley, and K. Knight.** 1993. Isolation of a herpes simplex virus type 1 mutant with a deletion in the virion host shutoff gene and identification of multiple forms of the *vhs* (UL41) polypeptides. J. Virol. **67:**7149–7160.
43. **Read, G. S., and N. Frenkel.** 1983. Herpes simplex virus mutants defective in the virion-associated shutoff of host polypeptides synthesis and exhibiting abnormal synthesis of α (immediate-early) viral polypeptides. J. Virol. **46:**498–512.
44. **Rice, S. A., M. C. Long, V. Lam, P. A. Schaffer, and C. A. Spencer.** 1995. Herpes simplex virus immediate-early protein ICP22 is required for viral modification of host RNA polymerase II and establishment of the normal viral transcription program. J. Virol. **69:**5550–5559.
45. **Rice, S. A., M. C. Long, V. Lam, and C. A. Spencer.** 1994. RNA polymerase II is aberrantly phosphorylated and localized to viral replication compartments following herpes simplex virus infection. J. Virol. **68:**988–1001.
46. **Richter-Cook, N. J., T. E. Dever, J. O. Hensold, and W. C. Merrick.** 1998. Purification and characterization of a new eukaryotic protein translation factor. Eukaryotic initiation factor 4H. J. Biol. Chem. **273:**7579–7587.
47. **Roizman, B., and D. M. Knipe.** 2001. Herpes simplex viruses and their replication, p. 2381–2398. *In* D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb, M. A. Martin, B. Roizman, and S. E. Strauss (ed.), Fields virology, 4th ed. Lippincott Williams & Wilkins, Philadelphia, Pa.
48. **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.
49. **Salio, M., M. Cella, M. Suter, and A. Lanzavecchia.** 1999. Inhibition of dendritic cell maturation by herpes simplex virus. Eur. J. Immunol. **29:**3245–3253.
50. **Samady, L., E. Costigliola, L. MacCormac, Y. McGrath, S. Cleverley, C. E. Lilley, J. Smith, D. S. Latchman, B. Chain, and R. S. Coffin.** 2003. Deletion of the virion host shutoff protein (vhs) from herpes simplex virus (HSV) relieves the viral block to dendritic cell activation: potential of vhs$^-$ HSV vectors for dendritic cell-mediated immunotherapy. J. Virol. **77:**3768–3776.
51. **Sandri-Goldin, R. M.** 1998. Interactions between a herpes simplex virus regulatory protein and cellular mRNA processing pathways. Methods **16:**95–104.
52. **Sato, H., L. D. Callanan, L. Pesnicak, T. Krogmann, and J. I. Cohen.** 2002. Varicella-zoster virus (VZV) ORF17 protein induces RNA cleavage and is critical for replication of VZV at 37°C but not 33°C. J. Virol. **76:**11012–11023.
53. **Sciabica, K. S., Q. J. Dai, and R. M. Sandri-Goldin.** 2003. ICP27 interacts with SRPK1 to mediate HSV splicing inhibition by altering SR protein phosphorylation. EMBO J. **22:**1608–1619.
54. **Smibert, C. A., and J. R. Smiley.** 1990. Differential regulation of endogenous and transduced β-globin genes during infection of erythroid cells with a herpes simplex virus type 1 recombinant. J. Virol. **64:**3882–3894.
55. **Smiley, J. R., M. M. Elgadi, and H. A. Saffran.** 2001. Herpes simplex virus vhs protein. Methods Enzymol. **342:**440–451.
56. **Smiley, J. R., B. Panninng, and C. A. Smibert.** 1990. Regulation of cellular genes by HSV products, p. 151–179. *In* E. K. Wagner (ed.), Herpesvirus and its transcription. CRC Press, Boca Raton, Fla.
57. **Song, B., K. C. Yeh, J. Liu, and D. M. Knipe.** 2001. Herpes simplex virus gene product required for expression of G1-phase functions. Virology **290:**320–328.
58. **Sorenson, C. M., P. A. Hart, and J. Ross.** 1991. Analysis of herpes simplex virus-induced mRNA destabilizing activity using an in vitro mRNA decay system. Nucleic Acids Res. **19:**4459–4465.
59. **Spencer, C. A., M. E. Dahmus, and S. A. Rice.** 1997. Repression of host RNA polymerase II transcription by herpes simplex virus type 1. J. Virol. **71:**2031–2040.
60. **Strelow, L., T. Smith, and D. Leib.** 1997. The virion host shutoff function of herpes simplex virus type 1 plays a role in corneal invasion and functions independently of the cell cycle. Virology **231:**28–34.
61. **Strelow, L. I., and D. A. Leib.** 1996. Analysis of conserved domains of UL41 of herpes simplex virus type 1 in virion host shutoff and pathogenesis. J. Virol. **70:**5665–5667.
62. **Strelow, L. I., and D. A. Leib.** 1995. Role of the virion host shutoff (*vhs*) of herpes simplex virus type 1 in latency and pathogenesis. J. Virol. **69:**6779–6786.
63. **Strom, T., and N. Frenkel.** 1987. Effects of herpes simplex virus on mRNA stability. J. Virol. **61:**2198–2207.
64. **Suzutani, T., M. Nagamine, T. Shibaki, M. Ogasawara, I. Yoshida, T. Daikoku, Y. Nishiyama, and M. Azuma.** 2000. The role of the UL41 gene of herpes simplex virus type 1 in evasion of non-specific host defence mechanisms during primary infection. J. Gen. Virol. **81:**1763–1771.
65. **Taddeo, B., A. Esclatine, W. Zhang, and B. Roizman.** 2003. The stress-inducible immediate-early responsive gene IEX-1 is activated in cells infected with herpes simplex virus 1, but several viral mechanisms, including 3′ degradation of its RNA, preclude expression of the gene. J. Virol. **77:**6178–6187.
66. **Tigges, M. A., S. Leng, D. C. Johnson, and R. L. Burke.** 1996. Human herpes simplex virus (HSV)-specific CD8+ CTL clones recognize HSV-2-infected fibroblasts after treatment with IFN-gamma or when virion host shutoff functions are disabled. J. Immunol. **156:**3901–3910.
67. **Trgovcich, J., D. Johnson, and B. Roizman.** 2002. Cell surface major histocompatibility complex class II proteins are regulated by the products of the $\gamma_1$34.5 and U_L41 genes of herpes simplex virus 1. J. Virol. **76:**6974–6986.
68. **Walker, J., K. A. Laycock, J. S. Pepose, and D. A. Leib.** 1998. Postexposure vaccination with a virion host shutoff defective mutant reduces UV-B radiation-induced ocular herpes simplex virus shedding in mice. Vaccine **16:**6–8.
69. **Walker, J., and D. A. Leib.** 1998. Protection from primary infection and establishment of latency by vaccination with a herpes simplex virus type 1 recombinant deficient in the virion host shutoff (vhs) function. Vaccine **16:**1–5.
70. **Zelus, B. D., R. S. Stewart, and J. Ross.** 1996. The virion host shutoff protein of herpes simplex virus type 1: messenger ribonucleolytic activity in vitro. J. Virol. **70:**2411–2419.
71. **Zhou, C., and D. M. Knipe.** 2002. Association of herpes simplex virus type 1 ICP8 and ICP27 proteins with cellular RNA polymerase II holoenzyme. J. Virol. **76:**5893–5904.