EARLY SHUTOFF OF HOST PROTEIN SYNTHESIS IN CELLS INFECTED WITH HERPES SIMPLEX VIRUSES

J. MATIS*, M. KÚDELOVÁ

Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 46 Bratislava, Slovak Republic

Received September 3, 2001; accepted October 26, 2001

Summary. – Herpes simplex viruses 1 (HSV-1) and 2 (HSV-2) are capable of suppressing the host cell protein synthesis even without viral gene expression. This phenomenon is known as the early shutoff or as the virion-associated host shutoff (*vhs*) to emphasize that it is mediated by a component of infecting virions which is a product of the UL41 (*vhs*) gene. The UL41 encoded protein is a functional tegument protein also present in light (L) particles and is not essential for virus replication. The major product of UL41 gene is a 58 K phosphoprotein. At least two forms of UL41 protein differing in the extent of phosphorylation are present in HSV-1-infected cells. HSV-2 compared to HSV-1 strains display a stronger vhs phenotype. However, in superinfection experiments the less strong vhs phenotype is dominant. UL41 protein triggers disruption of polysomes and rapid degradation of all host and viral mRNAs and blocks a reporter gene expression without other HSVs proteins. The available evidence suggests that UL41 protein is either itself a ribonuclease (RNase) or a subunit of RNase that contains also one or more cellular subunits. UL41 protein is capable of interacting with a transactivator of an α-gene, the α-transinducing factor (α-TIF). Interaction of UL41 protein with α-TIF down regulates the UL41 (*vhs*) gene activity during lytic infection. The possible role of other viral proteins in the shutoff is discussed.

Key words: HSV-1; HSV-2; host protein synthesis; early shutoff; *vhs* gene; vhs protein

Introduction

Over 100 different herpesviruses have been described in various animal species. They are readily identified by the distinctive architecture of the virion (Horne and Wildy, 1961). In the center of the virion (virus core) is located the genome. The genome is enclosed in an icosahedral capsid assembled from 162 capsomers composed of a number viral structural proteins. The capsid with enclosed viral DNA (nucleocapsid) is embedded in an apparently amorphous matrix termed the tegument. The outer surface of the tegument is enclosed in a viral envelope, a lipid bilayer of host origin containing at least 12 different virus membrane proteins, predominantly glycoproteins.

Herpesvirus replication has been most extensively studied on HSVs. The HSV-1 genome (dsDNA) consisting of two regions, short (US) and long (UL), and flanked by inverted repeats (IRs), is 150 kbp in size and has a potential to encode at least 70 polypeptides designated ICPs (infected cell proteins) (Wadsworth *et al.*, 1975). The gene expression of HSV-1 is highly ordered (Honess and Roizman, 1974). HSV-1 genes have been partitioned into three large groups, namely α, β and γ, based on the time of appearance and requirements for expression. The α (immediate-early) genes are by

*E-mail: virumati@nic.savba.sk; fax: +4217-54774284.

Abbreviations: α-TIF = α-trans-inducing factor; BoHV-1 = bovine herpesvirus 1; dsDNA, dsRNA = double-stranded DNA, RNA; eIF-2 = translation initiation factor; EHV-1 = equine herpesvirus 1; GADD = growth arrest and DNA damage; HSV-1 = herpes simplex virus 1; HSV-2 = herpes simplex virus 2; HSVs = HSV-1 and/or HSV-2; ICP = infected cell protein; IR = inverted repeat; IRES = internal ribosome entry site; L particles = light particles; MHC = major histocompatibility complex; Oct-1 = octamer-binding protein; p.i. = post infection; PKR = protein kinase R; PRV = pseudorabies virus; PP1 = protein phosphatase 1; TAP = peptide transporter associated with antigen presentation; ts = temperature-sensitive; UL = long unique region; US = short unique region; vhs = virion-associated host shutoff; VZV = varicella-zoster virus

definition expressed in the absence of viral protein synthesis. Five α proteins, namely ICP0, ICP4, ICP22, ICP27, and ICP47 have been identified. Except ICP47 the α proteins function as regulators and their maximum synthesis occurs at 2–4 hrs post infection (p.i.).

The expression of β (early) genes requires the synthesis of α proteins. β proteins reach maximum synthesis at 5–7 hrs p.i. and are directly or indirectly involved in viral DNA synthesis. β proteins include five proteins (enzymes) involved in nucleotide metabolism, e.g. thymidine kinase (ICP36) and ribonucleotide reductase (ICP6), and seven proteins required for viral DNA replication (e.g. DNA polymerase and the major DNA binding protein (ICP8)). β proteins have been further partitioned into β_1 and β_2 subclasses according to their kinetics of synthesis. β_1 proteins (e.g. ICP6 and ICP8) are synthesized earlier, while β_2 proteins (e.g. ICP36 and DNA polymerase) are synthesized later.

The γ (late) genes are expressed at 12–16 hrs p.i. and are also partitioned into two subclasses, γ_1 and γ_2. γ_1 genes, expressed earlier (e.g. major capsid protein ICP5), are not affected by inhibitors of DNA synthesis, while γ_2 genes, expressed later (e.g. glycoprotein C), depend strictly on viral DNA synthesis.

The synthesis of γ proteins, predominantly structural ones, is a characteristic feature of productive virus replication. The fact that the virus growth *in vitro* is accompanied by an overall inhibition of host protein synthesis is a long-standing finding (Roizman *et al.*, 1965; Sydiskis and Roizman, 1966, 1967). However, experiments with the UV irradiated virus or with the drugs precluding expression of infecting virions revealed that some strains of HSVs possess the ability to suppress host protein synthesis even in the absence of viral protein synthesis. This phenomenon has become known as early shutoff or virion-associated shutoff of host protein synthesis to emphasize that it is mediated by a component of infecting virions. The aim of this review is to summarize our knowledge about the nature of the viral component responsible for the early shutoff as well as to gain insight into the function of this component in virus replication. It is recommended to follow Fig.1 in reading the review.

Experiments leading to discovery of *vhs* gene function

Several early findings revealed that shortly after infection with HSVs cellular polysomes disaggregate (Roizman *et al.*, 1965; Sydiskis and Roizman, 1966, 1967) and host protein synthesis begins to decline irreversibly (Powell and Courtney, 1975; Fenwick and Walker, 1978). Furthermore, HSV-1 causes a degradation of host RNA (Nishioka and Silverstein, 1977, 1978a), a dissociation of cellular mRNA from polysomes (Nishioka and Silverstein, 1978) and an inhibition of cellular DNA and RNA synthesis (Roizman *et al.*, 1965; Stenberg and Pizer, 1982). HSV-2 strains shut off cellular protein synthesis more rapidly and more completely than do HSV-1 strains (Everly *et al.*, 1977, 1999; Pereira *et al.*, 1977). To explain this difference it has been suggested that viral protein synthesis is needed for inhibition of host protein synthesis with HSV-1 but not with HSV-2. In the case of HSV-2 a virion component was suggested to be involved in the shutoff (Sydiskis and Roizman, 1966; Fenwick and Walker, 1978). In efforts to determine how the shutoff phenomena induced with HSV-1 and HSV-2 differ, Hill *et al.* (1983) have used FL cells which proved to be useful as a model host system for studying HSVs-induced changes in cellular macromolecular synthesis (Nishioka and Silverstein, 1977, 1978a, 1978b). When induced with dimethylsulfoxide, FL cells are capable of producing large amounts of globin mRNA and globin (Ross *et al.*, 1972). Hill *et al.* (1983) have found striking differences in the host shutoff functions of HSV-1 strain KOS and HSV-2 strain 186. In FL cells, HSV-2 inhibited globin synthesis by more than 80% at 30 mins p.i. This inhibition was extremely resistant to a prior UV irradiation of virions. Also experiments, in which actinomycin D or me_3-psoralen treatment was employed to block viral transcription, produced similar results. However, the HSV-1-induced shutoff was sensitive to such treatments. These results (Hill *et al.*, 1983) strongly suggested that HSV-1 requires transcription of viral genes to arrest cellular protein synthesis, while HSV-2 utilizes a virion-associated component for this purpose. However, other studies did not confirm this hypothesis. Two distinct shutoff processes have been described in HSV-1-infected cells (Nishioka and Silverstein, 1978a). The host polysome disaggregation in FL cells was found to be independent of viral gene expression, indicating that a virion-associated component was responsible for this phenomenon. However, this disaggregation did not arrest the synthesis of host proteins. The inhibition of host protein synthesis required expression of the viral genome accompanied by the degradation of cellular mRNA. Fenwick and Clark (1982), using the *ts*B7 temperature-sensitive mutant of HSV-1 strain HFEM and Vero cells, have also observed two distinct stages of virus-induced shutoff of host protein synthesis. They have designated the shutoff requiring expression of viral genes as "delayed" and the shutoff caused by a virion component as "early". The early shutoff function was temperature-sensitive in both *ts*B7 mutant and RC2 revertant as well as in an unrelated HSV-1 strain F, being active at 34°C but not at 39°C. The early shutoff function of HSV-2 strain G was temperature-insensitive. Thus, the virion-associated polysome disaggregation function observed at 37°C by Nishioka and Silverstein (1978a), which had little effect on host protein synthesis, could represent an attenuated form of the early shutoff. It has been concluded that similar shutoff

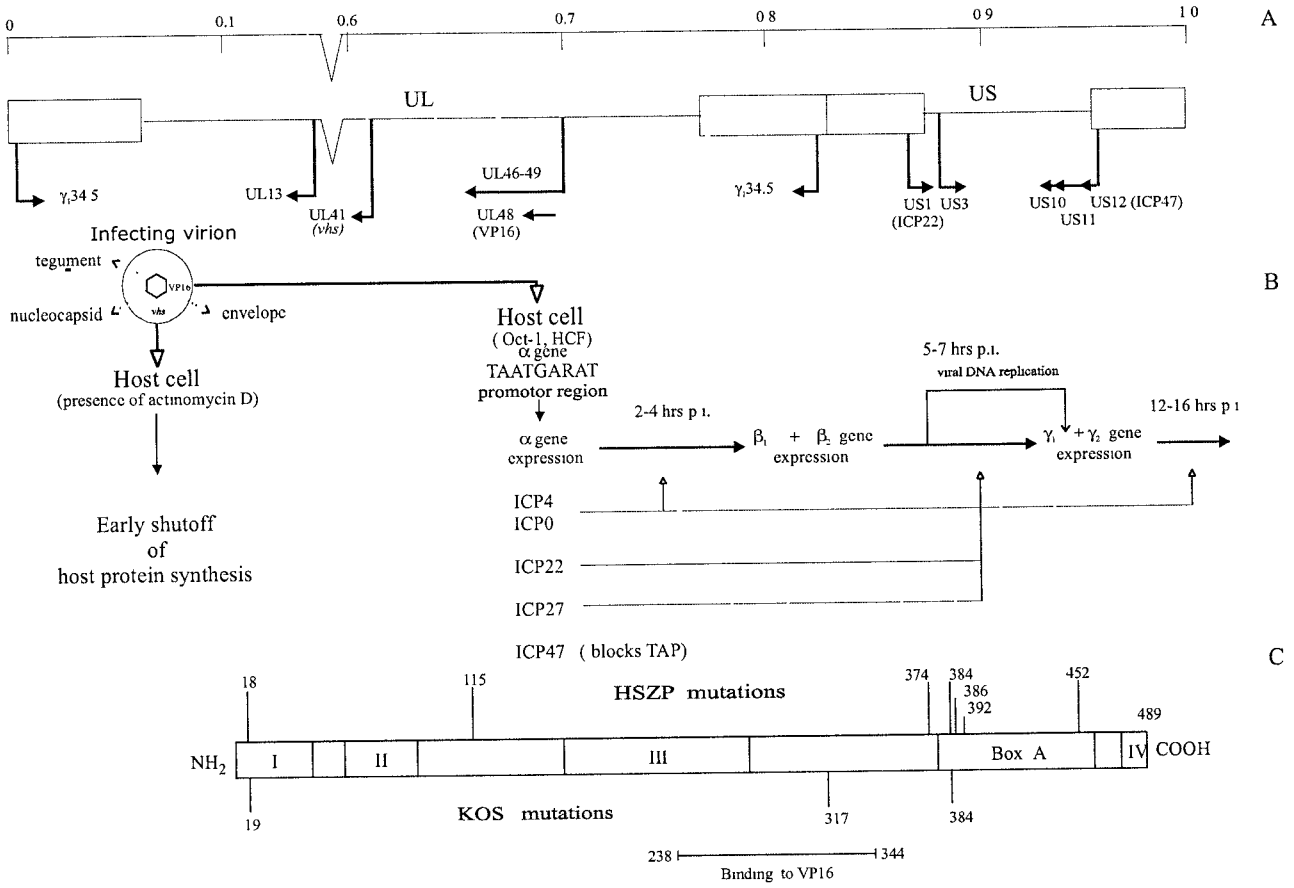

Fig. 1

HSV-1 gene transcription regulation and functional map of vhs protein

(A) The HSV-1 genome in prototype orientation with UL and US regions flanked by IRs marked by boxes. Size and orientation of viral genes of concern are indicated by arrows. The upper scale represents fractional map unit. (B) The HSVs infection of a permissive cell. (C) The distribution of amino acid mutations in HSZP and KOS strains within the functional map of vhs protein of 17 strain. The conserved regions with amino acid sequence invariance over 25% among five alphaherpesviruses (boxes I to IV) and the less conserved region with 11% invariance (box A) (Vojvodová *et al.*, 1997).

components are present in HSV-1 and HSV-2 virions, differing only in thermal stability.

Information resulting from studies of *vhs* gene mutants

The finding that the infection of cells with HSV-2 strains results in a more rapid shutoff compared to HSV-1 strains (Everly *et al.*, 1997; Pereira *et al.*, 1977) supported the hypothesis that the shutoff function is determined by the genetic makeup of infecting virus. Using HSV-1 x HSV-2 recombinants, the accelerated shutoff has been mapped to a distinct region of the HSV-2 genome, spanning map coordinates 0.52 to 0.59 (Fenwick *et al.*, 1979; Morse *et al.*, 1978). Using 5-bromo-2'-deoxyuridine, several HSV-1 mutants exhibiting a defective vhs function were isolated (Read and Frenkel, 1983). The mutants were capable of proper entry into the cells, but unlike the parental wild-type (wt) virus, they failed to shut off host protein synthesis in the presence of actinomycin D. They were designated as vhs mutants. In the absence of actinomycin D, the vhs mutants induced a secondary (delayed) shutoff of translation of host mRNA, which required the synthesis of viral β and γ polypeptides. The synthesis of host proteins was turned off, albeit in a delayed and incomplete manner. It was found that in cells infected with a mutant designated as *vhs*1 both host and viral mRNAs were more stable than those in cells infected with wt virus (Schek and Bachenheimer, 1985; Kwong and Frenkel, 1987; Oroskar and Read, 1987). The vhs1 mutation affecting the shutoff of host protein synthesis, the degradation of host mRNA as well as the destabilization

of functional viral α, β and γ mRNAs mapped within a 265 bp region spanning map coordinates 0.604 to 0.606 of HSV-1 genome (Kwong *et al.*, 1988). Based on earlier transcriptional mapping and sequence analysis of HSV-1 strain 17 (Frink *et al.*, 1981; McGeoch *et al.*, 1988), the vhs1 point mutation (Thr214→Ile) lies within the vhs ORF which is predicted to encode a 489 amino acid protein of 58 K. To identify the vhs protein, Smibert *et al.* (1992) have raised a rabbit antiserum against a synthetic peptide corresponding to amino acids 333 to 347. They were able to identify a single 58 K phosphoprotein from HSV-1 infected cells and virions. In further studies two electrophoretically distinguishable forms of the protein were identified in cells infected with either wt HSV-1 or mutant vhs1 (Read *et al.*, 1993). wt HSV-1 produced a major 58 K protein as well as a less abundant 59.5 K protein, while vhs1 produced 57 K and 59K proteins which were approximately equally abundant. For both viruses, both forms of the protein were phosphorylated, although to different extent. Both vhs proteins were found in infected cells but only the faster migrating, less phosphorylated form was incorporated into virions. Thus, the identification of multiple forms of vhs protein suggested that posttranslational processing may affect its packaging into virions as well as its ability to induce mRNA degradation.

vhs gene homologs have been also identified in the genomes of equine herpesvirus 1 (EHV-1), varicella-zoster virus (VZV) and pseudorabies virus (PRV). However, nothing is known about the expression of these *vhs* gene homologs or the activities of their putative products (Berthomme *et al.*, 1993; Feng *et al.*, 1996). It has been recently reported that bovine herpesvirus 1 (BoHV-1) exhibits a vhs-like activity (Hinkley *et al.*, 2000). The putative BoHV-1 ORF contained the four conserved regions of vhs protein of alphaherpesviruses (see below).

Localization of vhs protein in virion

The first evidence that the inhibitor responsible for the early shutoff of host macromolecular synthesis is a constituent of the infecting particles present in the inoculum was given by Fenwick and Walker (1978). The authors have found correlation between the infectivity of virus inoculum and the early inhibition of host protein synthesis by diluting the inoculum. Furthermore, the experiment in which crude virus inoculum was centrifuged at high speed and the resulting supernatant was used as inoculum showed that the inhibition was not mediated by a soluble factor remaining in the supernatant. This finding has been later confirmed by others with purified virus used as inoculum (Smibert *et al.*, 1992; Read *et al.*, 1993; Matis and Krivjanská, 1994). The assumption that *vhs* gene product represents a tegument protein (Kwong *et al.*, 1988) has been excellently confirmed by characterization of non-infectious HSV-1-related particles designated as "light" (L) particles (Szilágy and Cunningham, 1991). The L-particles were isolated by centrifugation through a Ficoll gradient, a procedure previously involved in the purification and separation of enveloped and unenveloped HSV-1 particles (Matis *et al.*, 1975). L particles lack the nucleocapsid but contain tegument as well as envelope and possess vhs activity. Thus, they are as efficient as virions to deliver functional proteins into host cells. Examination of the polypeptide profiles of virions and L-particles deenveloped by non-ionic detergent treatment revealed the presence of vhs protein and thus confirmed its tegument localization (McLauchlan and Rixon, 1992; McLauchlan *et al.*, 1992).

Interference phenomenon

Hill *et al.* (1985) have reported that a HSV-1 virion component that delayed the shutoff could interfere with early shutoff induced by a HSV-2 strain. It has been postulated that the observed inhibition of HSV-2-induced early shutoff by HSV-1 results from competition between HSV-1 and HSV-2 virion-associated components for target sites within the cell. However, such a predominance of the weak shutoff function was not confirmed in experiments with a recombinant virus containing both weak and strong alleles of UL41 gene (Fenwick and Everett, 1990). This observation indicates that the spatial relationship of both UL41 gene products can play a role in the interference. The finding of Hill *et al.* (1985) was also confirmed with HSV-1 strains (Matis and Krivjanská, 1988). It was shown that the HSV-1 HSZP strain, defective in the early shutoff of host protein synthesis (Matis and Szántó, 1985), was still capable of interfering with the early shutoff of host protein synthesis induced by HSV-1 KOS strain, even when heat-inactivated or neutralized by antibody (Matis and Krivjanská, 1988; Matis *et al.*, 1992). The UL41 gene of HSZP strain was sequenced and compared with the corresponding sequences of KOS and 17 strains (Vojvodová *et al.*, 1997). Two KOS-specific amino acid mutations were identified. One of them mapped to the region responsible for the binding of vhs protein to α-TIF (VP16). Therefore, this mutation may be responsible for the pronounced shutoff activity of KOS strain (see below). It has been reported that all mutants mapping to regions conserved among the vhs homologs of alphaherpesviruses (regions I to IV and box A) as well as the insertion mapping just six residues apstream of box A (residue 374) inactivates the function of a reporter gene (Berthomme *et al.*, 1993; Jones *et al.*, 1995). Moreover, Everett and Fenwick (1990) have presented evidence that

the C-terminal part of the UL41 protein is essential for its function. They have showed that the inability of the HSV-2 HG52 strain to suppress host protein synthesis was a consequence of a frameshift mutation in the UL41 coding region (amino acid 343) leading to the production of a truncated UL41 protein (406 instead of 492 amino acids). HG52 strain failed also to inhibit the shutoff induced by other HSV-2 strains. Assuming that the loss of interference ability in mixed infections reflects the truncated form of the HG52 vhs protein, the finding that HSZP strain is effective at interfering but defective in the shutoff function allows to propose that mutations mapping to the A box and to amino acid 374 may contribute to failure of the HSZP shutoff function. It has also been shown that the low pH-inactivated HSZP virions are able to interfere with the shutoff function of the KOS strain when their penetration into host cells is induced by PEG treatment (Matis *et al.*, 1999). However, the HSZP strain failed to interfere when inactivated with zinc ions or purified from cells treated with 2-deoxy-D-glucose (Matis and Krivjanská, 1994). This finding supported the idea that a specific interaction between HSZP virions and the cell surface may be responsible for the interference phenomenon. In this connection it is interesting that in neomycin-treated Hep-2 cells the shutoff did not occur (Garcin *et al.*, 1990). As neomycin is an inhibitor of the inositol phospholipid pathway (Reid and Gajjar, 1987), this finding suggests a possible contribution of the signal-transduction mechanism to the shutoff in HSV-1-infected Hep-2 cells. Furthermore, the ts 1204 mutant of HSV-1 that is not able to penetrate the plasma membrane is able to activate expression of a cellular gene by specific binding to the cell surface (Preston, 1990). Nevertheless, further investigations into the mechanism of the interference phenomenon are needed.

Participation of viral proteins in *vhs* protein function

Approximately 30 distinct structural proteins have been identified in the purified HSVs virions (Spear and Roizman, 1972). Over half of them are believed to be components of the tegument, which was originally defined as an amorphous, electron-dense region located between the nucleocapsid and viral envelope (Roizman and Furlong, 1974). Four viral proteins which represent the bulk of the tegument mass are encoded by genes located in a consecutive stretch in the UL region of HSV-1 genome. UL46, UL47, UL48, and UL49 genes encode proteins designated as VP11/12 (Zhang and McKnight, 1993), VP13/14 (McLean *et al.*, 1990), VP16 (Campbell *et al.*, 1984) and VP22 (Elliott and Meredith, 1992), respectively. In addition, at least UL13 gene encoding another tegument protein (protein kinase) should be mentioned (Overton *et al.*, 1992). All these proteins are phosphoproteins (Lemaster and Roizman, 1980), which may together with vhs protein undergo additional posttranslational modification.

Several reports have suggested that the function of vhs protein may be modified by other viral proteins. Namely, vhs protein may interact with VP16, a product of UL48 gene (Smibert *et al.*, 1994). VP16 (also known as Vmw65, ICP25 and α-TIF) is a component of the virion tegument and transactivators of α genes (Batterson and Roizman, 1983). VP16, an abundant 65 K virion phosphoprotein is synthesized late in the infection and is subsequently packaged into virions (Ace *et al.*, 1988). VP16 possesses an exceptionally strong acidic transcriptional activation domain (Sadowski *et al.*, 1988) and targets DNA through the TAATGARATT consensus sequence which is found in the immediate-early control regions of HSVs (Kristie and Roizman, 1984; Gaffney *et al.*, 1985). The low affinity of VP16 to the consensus sequence is significantly enhanced through association with at least two cellular factors; the octamer-binding protein (Oct-1) and HCF, also designated as VCAF-1 or C1.

Oct-1 binds directly to the consensus sequence, while HCF binds directly to VP16 and mediates its stable association with DNA-bound Oct-1 (Gerster and Roeder, 1988; Stern *et al.*, 1989; Kristie and Sharp, 1993). The transcriptional activation of α genes by VP16 greatly increases the probability that cells infected with a single virus particle will enter the productive cycle. Once engaged, the lytic program is self-sustaining, as α proteins suffice to maintain transcription of α genes and induce expression of the viral early and late genes (O'Hare and Hayward, 1985; Ace *et al.*, 1989; Cai and Schaffer, 1992). Thus VP16 plays a transient regulatory role during the earliest stages of infection. Mutations that inactivate the transcriptional activation function of VP16 result in a reduced level of α gene expression during a low-multiplicity infection and increase particle-to-PFU ratio (Ace *et al.*, 1989; Smiley and Duncan, 1997). It has been demonstrated that a small modular domain in vhs protein encompassing amino acids 310 to 330 is sufficient for directing a stable interaction with VP16 and that tryptophan 321 is critical for this interaction (Schmelter *et al.*, 1966). vhs protein binds to the promoter recognition domain of VP16 and such a complex is not capable of interacting with Oct-1, HCF and DNA (Smibert *et al.*, 1994). The interaction of VP16 with vhs protein down regulates the vhs protein activity during lytic infection, thereby allowing the accumulation and translation of viral mRNAs. The finding that VP16 down regulates the vhs protein activity is striking because the levels of VP16 in the virion tegument greatly exceed those of vhs protein (McLauchlan *et al.*, 1992). In spite of this, HSVs virions exhibit strong vhs protein activity.

One explanation may be that host VP16-binding proteins such as HCF displace vhs protein from VP16 shortly after infection because host factors bind VP16 more avidly than does vhs protein (Smibert *et al.*, 1994). Another possibility is that the vhs protein-VP16 interaction is modulated by products of other HSVs genes. One candidate was UL13 gene predicted to encode a tegument-associated protein kinase (Overton *et al.*, 1992). It has been reported that UL13 gene-deficient virus mutants display a null vhs phenotype, even though the mutant virions contain normal amounts of vhs protein (Overton *et al.*, 1994). The authors have shown that UL13 gene-deficient mutants exhibit different patterns of protein phosphorylation as compared with wt virus when infected cells were labeled with [^{32}P]-orthophosphate or when lysates of infected cells and virions were subjected to *in vitro* phosphorylation. However, vhs protein could still be phosphorylated in lysates of UL13-deficient virions. They have concluded that the UL13 gene is necessary to produce the early shutoff, but it seems unlikely that the role of UL13 gene is to activate the *vhs* gene product by phosphorylation. Thus these findings did not support the hypothesis that the UL13 protein kinase may be responsible for disruption of the vhs protein-VP16 complex during virion uncoating by phosphorylating vhs protein or VP16 (Lam *et al.*, 1996). It has been reported that the UL13 protein kinase of HSV-1 appears to mediate the phosphorylation of several viral proteins including the regulatory α protein ICP22 (Purves and Roizman, 1992; Purves *et al.*, 1993; Coulter *et al.*, 1993). The gene encoding ICP22 produces two of mRNAs. The longer mRNA encodes ICP22 and the shorter one, designated as US1.5 mRNA encodes approximately the C-half of ICP22 (Carter and Roizman, 1996). An extensive posttranslational modification of ICP22 includes phosphorylation by UL13 and US3 viral protein kinases and by casein kinase II (Purves *et al.*, 1993; Mitchell *et al.*, 1994). Further studies have clearly revealed that the failure of UL13 gene-deficient mutant viruses to exert the shutoff of host protein synthesis shortly after infection is due to the failure of the UL13 protein kinase to phosphorylate the ICP22 gene products (Ng *et al.*, 1997). Thus, the regulatory effect of UL13 protein kinase on the vhs protein is indirect and not limited to direct interaction of these two viral proteins. UL13 protein kinase and ICP22 which positively regulate the vhs protein function are also involved in alteration of the phosphorylation state of RNA polymerase II that specifically represses cellular gene transcription and promotes viral late transcription (Rice *et al.*, 1995; Long *et al.*, 1999). They are also involved in the posttranslational modification of a cellular protein p60 in restrictive rabbit skin cells (Bruni *et al.*, 1999). Moreover, the UL13 protein kinase is involved in the posttranslational modification of the cellular elongation factor 1 delta (Kawaguchi *et al.*, 1999).

The UL41 gene belongs to the category of early/late genes and, as already mentioned, the vhs protein made late in the infection is packaged in the tegument of the virion. In this connection a question arisen as to why vhs protein made late in the infection did not exert the shutoff of protein synthesis in cells in which it had been made. Lam *et al.* (1996) have proposed that this function is blocked by VP16. Also the possibility that the product of the $\gamma_1$34.5 gene may be involved in this phenomenon was tested. The $\gamma_1$34.5 gene maps to IRs flanking the UL region and is therefore present in two copies per genome. The $\gamma_1$34.5 gene of HSV-1 encodes a protein capable of precluding the total shutoff of protein synthesis after the onset of viral DNA synthesis in infected cells of human origin (Chou and Roizman, 1992). The shutoff is completed by 13 hrs p.i. and is related to phosphorylation of the α subunit of the translation initiation factor eIF-2 by the double-stranded RNA (dsRNA)-dependent protein kinase R (PKR) (Chou *et al.*, 1994, 1995). Poon and Roizman (1997) tested the hypothesis that in human cells the shutoff of protein synthesis is mediated by vhs protein and the function of the $\gamma_1$34.5 protein is merely to block the function of vhs protein synthetised late in the infection. However, the experiments revealed that the functions of UL41 and $\gamma_1$34.5 genes were independent of each other. The authors have come to the conclusion that $\gamma_1$34.5 protein blocks the host response to the infection by affecting somehow the ability of activated PKR to phosphorylate the translational initiation factor eIF-2. Other studies have shown that the C terminus of $\gamma_1$34.5 protein binds to the protein phosphatase 1α (PP1) and redirects it to dephosphorylate eIF-2. Moreover, the amino acid sequence of $\gamma_1$34.5 protein which interacts with the phosphatase is high homologous to a domain of a conserved mammalian protein known as GADD34 (growth arrest and DNA damage) protein (Zhan *et al.*, 1994). The effectiveness of the $\gamma_1$34.5-PP1 complex is apparent from the observation that the rate of dephosphorylation of eIF-2 in cells infected with wt virus is more than 1000 times higher than that of uninfected cells or cells infected with the $\gamma_1$34.5 gene-deficient virus (He *et al.*, 1997). By serial passaging a $\gamma_1$35.4 gene-deficient mutant Mohr and Gluzman (1996) have isolated compensatory mutants capable of sustained protein synthesis. They had a deletion in ICP47 gene resulting in a juxtaposition of the ICP47 gene promoter next to US11, a late (γ_2) viral gene. Thus the deletion changed the kinetics of expression of US11 gene from late (γ_2) to early (β) type. Moreover, a later study (He *et al.*, 1997 has shown that PKR is activated in cells infected with compensatory mutants, suggesting that HSV-1 disposes of a mechanism capable of blocking the shutoff of protein synthesis by the activated PKR. The activation of this mechanism by the compensatory mutation results in the expression of a factor which precludes the phosphorylation of eIF-2 (Cassady *et al.*, 1998). US11 protein is one of the most abundant late viral proteins which

binds mRNA, accumulates in nucleoli, associates with ribosomes and is also packaged in virions (Puvion-Dutilleul *et al.,* 1985; Maclean *et al.,* 1987; Roller and Roizman, 1992). Moreover, it may have novel functions not readily apparent from its location in infected cells (Diaz *et al.,* 1996). Thus US11 protein can exhibit multiple functions and further studies are needed to clarify the mechanism by which it blocks PKR.

$\gamma_1$34.5 gene, which enables replication and spread of the virus in the central nervous system (Chou *et al.,* 1990), is not highly conserved among herpesviruses (Chou and Roizman, 1994). Therefore, it can be expected that viruses lacking $\gamma_1$34.5 gene have evolved alternative mechanisms to deal with this host response to infection. Taken into the consideration that GADD34 protein performs a similar function in uninfected cells under conditions of stress, it is possible that in the course of evolution, $\gamma_1$34.5 gene protein product acquired the C-terminal domain of GADD34 protein (Cassady *et al.,* 1998). Thus, the evolution of $\gamma_1$34.5 gene may be a relatively recent phenomenon.

Target of vhs protein activity

As already mentioned above, a remarkable feature of the shutoff mediated by the vhs protein is its specificity for mRNA. In addition to triggering degradation of cellular mRNAs, vhs protein also significantly destabilizes HSV mRNAs of all three temporal classes (Kwong and Frenkel, 1987). It has been demonstrated that the degradation of sequences at or near the 5'-end of mRNAs is an early step in the vhs protein-induced decay (Karr and Read, 1999). However, the factors which direct the vhs protein to mRNAs in preference to other RNAs are unknown. The poly(A) tail is not required for an mRNA to become the target of the vhs protein activity *in vitro* and the ribonuclease (RNase) activity that was observed in virion extracts cleaved both capped and uncapped RNAs (Zelus *et al.,* 1996). It is possible that vhs protein distinguishes mRNAs from other RNAs on the basis of the fact that they are being translated or are potentially translatable. vhs protein is capable of inducing endoribonucleolytic cleavage of a variety of reporter mRNAs when expressed as the only HSVs protein in a rabbit reticulocyte lysate (RRL) *in vitro* translation system (Pak *et al.,* 1995; Elgadi *et al.,* 1999) and displays a still significant amino acid sequence similarity to the fen-1 family of nucleases that are involved in DNA replication and repair in eukaryotes and archaebacteria (Doherty *et al.,* 1996). The currently available evidence strongly suggests that vhs protein is either itself an RNase or else a subunit of an RNase that also includes one or more cellular subunits (Lu *et al.,* 2001a). It has been currently demonstrated that a vhs1 mutant considered earlier to abolish vhs activity (see above) induces readily detectable endoribonuclease activity on RNA substrates bearing an internal ribosome entry site (IRES) of encephalomyocarditis (EMC) virus in the RRL assay system (Lu *et al.,* 2001b). In this connection there is an interesting possibility that a wt vhs protein utilizes normally both IRES-dependent and IRES-independent modes of substrate recognition, while the vhs1 mutation impairs selectively the latter mode. In the case the vhs1 mutant protein is capable of destabilizing IRES-bearing mRNAs also *in vivo* the vhs1 protein may offer an approach to studying the biological function of the minority of cellular mRNAs bearing IRES. The exact nature of the vhs protein activity and targeting can be clarified only by further genetic and biochemical characterization of this viral protein.

Conclusions

vhs protein is one of the viral proteins mediating evasion of host defense mechanism. The evasive functions of other proteins encoded by HSVs genomes have been demonstrated. A complex of glycoproteins E and I forms a Fc receptor on the surface of infected cells and virions which is capable of participating in bipolar bridging antiviral immunoglobulin G (Frank and Friedman, 1989). Also glycoprotein C which is a Cb3 receptor is capable of suppressing complement activity (McNearney *et al.,* 1987). Further mechanisms are exemplified by two α proteins. ICP47 which blocks the antigen presentation on the surface of infected cells and thus mediates the escape from cytotoxic T cells (Hill *et al.,* 1995) and ICP27 which causes the delayed shutoff host protein synthesis through the regulation of small nuclear ribonucleoprotein distribution (Phelan and Clements, 1998).

Studies on the vhs protein have provided important insight about how HSVs appropriate the host cytoplasm for its own use. The vhs protein function affects in infected cells all mRNAs. Therefore, vhs protein might be able to affect modification of various cellular as well as viral functions. An important role of vhs protein *in vivo* could be evasion from non-specific host defence mechanisms during the primary HSVs infection through (i) suppression of cytokine production in the infected cells and (ii) reduction of the anti-HSVs activity of interferons α and β. However, this evasion might be only a part of the vhs protein function, since vhs protein reduces MHC class I molecules on the plasma membrane of HSVs-infected cells and a *vhs* gene-deficient strain induces strong immunity as a vaccine strain (Hill *et al.,* 1994; Walker and Leib, 1998). UL41 gene seems to be one of the genes whose elimination from live HSVs vaccines would be useful. E.g., the deletion of ICP47 gene would ensure that the infected cell MHC Class 1 antigen presentation pathway stays intact and unimpaired. Deleting

the $\gamma_1$34.5 protein coding region would specifically abolish that genome's neuroinvasiveness/neurovirulence, while deleting the reading frame from UL41 gene would eliminate the early shutoff function. Such deletions would allow propagation of a vaccine strain in various cell lines (Subak-Sharpe and Dargan, 1998).

Acknowledgements. Our results quoted in this paper have been achieved in studies partially supported by grants Nos. 2/999512/92, 2/1205/95, 95/5305/025 and 2/1081/21 of the Scientific Grant Agency of Ministry of Education of Slovak Republic and Slovak Academy of Sciences.

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