

A Multiple Functional Protein: the Herpes Simplex Virus Type 1 Tegument Protein VP22*

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Abstract: The herpes simplex virus type 1 (HSV-1) VP22, is one of the most abundant HSV-1 tegument proteins with an average stoichiometry of 2 400 copies per virion and conserved among *alphaherpesvirinae*. Many functions are attributed to VP22, including nuclear localization, chromatin binding, microtubule binding, induction of microtubule reorganization, intercellular transport, interaction with cellular proteins, such as template activating factor I (TAF-I) and nonmuscle myosin II A (NMIIA), and viral proteins including tegument protein VP16, pUS9 and pUL46, glycoprotein E (gE) and gD. Recently, many novel functions performed by the HSV-1 VP22 protein have been shown, including promotion of protein synthesis at late times in infection, accumulation of a subset of viral mRNAs at early times in infection and possible transcriptional regulation function.

Key words: Herpes simplex virus type 1 (HSV-1); VP22; Intercellular trafficking; Protein interaction; Tegument protein.

Human herpes simplex virus type 1 (HSV-1), a nuclear replicating DNA virus, is a widespread human pathogen that causes a lytic infection in the mucosal epithelial cells and a life-long latent infection in neurons. HSV-1 has a distinctive feature of having more than 20 tegument proteins between its capsid shell and envelope (29). Tegument proteins have been shown to play a variety of roles in infection including

the regulation of viral and host gene expression and the promotion of virus assembly and egress. An essential tegument protein, VP22, the product of the UL49 gene of HSV-1, has received notable attention for its large number of molecules in each polypeptide per virion (2400 molecules/virion) (18). It is a highly basic, rich in proline residues, nuclear phosphoprotein (14, 16, 19), capable of forming higher-order structures consisting of dimers or tetramers (33). VP22 contains 301 amino acids and has a predicted molecular weight of 32 kDa (16). It is conserved within the alpha-herpesvirus family, and undergoes a number of post-translational modifications during infection, including nucleotidylation, ADP-ribosylation and phosphorylation by at least two cellular kinases, casein kinase II

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and protein kinase C (PKC), and one virion protein kinase UL13 (36).

A number of functions have been attributed to VP22 and these functions are determined by different regions of this protein (1). VP22 possesses at least two separate determinants of nuclear and nucleolar localization (1, 21) and the protein accumulates to high levels in the nuclei of either infected cells (36) or stable VP22 expression cell lines (37). In cells, VP22 is first localized in the cytoplasm, where it binds to microtubules and induces collapse of the cytoskeleton, creating a large perinuclear lump. After the nuclear membrane has disintegrated in early mitosis, VP22 translocates into the nucleus and binds to chromatin, where it remains until after completion of mitosis and during the next interphase (1, 28). A recent study showed that nuclear VP22 targeted unique subnuclear structures early (<6 hpi) during HSV-1 infection and reduced the number of non-ring nucleolin patterns that seemed to imply that VP22 might function to inhibit nucleolar disassembly, thus stabilizing these structures (26). A mutant HSV lacking VP22 was able to assemble enveloped virions that reached extracellular compartments. This mutant produced virions with reduced immediate-early proteins (ICP0 and ICP4) and displayed delayed viral protein synthesis (15). Another VP22 null mutant described recently displayed defects in reaching extracellular compartments and in cell-to-cell spread (8). These two VP22 null viruses also decreased packaging of both glycoproteins gD and gE (8, 15). Recently, it was reported that the expressions of ICP0, gD and gE were regulated by the level of phosphate presented on VP22 (39). VP22 interacts with tegument protein VP16, α -trans-inducing factor (α -TIF), and relocates it to a novel

macromolecular assembly in coexpressing cells (13). Thus, VP22 plays some role in the final assembly of HSV, although likely in a redundant fashion with other tegument proteins (37). VP22 also can incorporate the mRNA into the virion and transport the mRNA to uninfected cells (43). Another important role of VP22 is that it can interact with template activating factor I (TAF-I) and impair the nucleosome assembly on entering viral DNAs (50).

Moreover, VP22 has the capability to spread intercellularly to adjacent cells after infection or transfection solely with a plasmid encoding VP22 (8, 9). This property is maintained in proteins fused with VP22, which can be used to transfer potentially therapeutic proteins into target cells. Furthermore, recent reports showed that VP22 was involved in promotion of protein synthesis, accumulation of a subset of viral mRNAs (7), and in transcriptional regulation (55). All of those studies imply that VP22 is important to virus replication. However, the detailed roles of VP22 during HSV-1 infection remain unclear. Some of its functions are described below.

INTERCELLULAR TRAFFICKING

VP22 is an efficient intercellular transporter, able to transfer into neighboring cells and translocate to the nucleus of the target cell (9). After expression in transfection cells, VP22 protein is exported from transfected cells via a non-classical Golgi-independent pathway. After secretion, VP22 can be efficiently internalized into the neighboring non-transfected cells by a poorly understood mechanism. Indeed, VP22 has been used as a cell-penetrating peptide/protein (CPP) (42). Many experiments have demonstrated that VP22 could enhance intercellular trafficking of proteins

fused to its N-or C-terminus *in vitro* and *in vivo* (17, 52, 53), and trafficking of VP22 was not restricted to particular tissues and species (17, 52). Moreover, VP22-fusion proteins retained both VP22-mediated transport properties and the biophysiological functions of the linked passenger proteins, enhancing the therapeutic effects of transduced genes and proteins. This property has been observed with VP22-linked p27, p53, CD (cytosine deaminase), SuperCD, which is the fusion protein of yeast CD with yeast uracil-phosphoribosyltransferase, and human papillomavirus E2 for delivery to tumors (24, 25). The property was also found in the fusion protein of VP22 with green fluorescent protein (VP22-GFP) following adenovirus-mediated transgene delivery to the central nervous system (CNS) neurons and others have also reported that VP22-GFP has the property of intercellular trafficking (9, 11, 25). Furthermore, VP22 mediated intercellular trafficking of I κ B- α can efficiently inhibit both constitutive and inducible nuclear factor kappa B (NF- κ B) activation (47). VP22-mediated intercellular protein delivery is also useful in enhancing the efficacy of myocardial gene therapy (3, 4, 53) and the effects of gene delivery for somatic gene therapy of Duchenne muscular dystrophy (DMD) (53, 54). The fusion gene of VP22- β -galactosidase (β Gal) transfection resulted in higher amounts of protein expression compared with β Gal transfection, and this study showed that VP22 had a remarkable secretory ability in living cells (32). Preclinical evaluation using the thymidine kinase (TK)-VP22 fusion protein revealed an enhanced cytotoxicity *in vitro* and *in vivo* using mixed populations of wildtype and expressing cells (53). However, some authors reported that fusion of HSV TK to VP22 did not result in intercellular

trafficking of the protein (2). In fact, others could not detect VP22-GFP trafficking in living cells, only in fixed cells. So it was hypothesized that this difference resulted from a concentration effect or removal of interfering components (11, 17), and it was even suggested that VP22 nuclear homing in living cells was an experimental artifact (27). Therefore, existing studies of VP22 intercellular spread remain confusing and difficult to interpret, but it should be pointed out that some proteins are readily extracted during methanol fixation of cells and that covalently bound polypeptides of a size nearly equivalent to or larger than VP22 could be expected to change the properties of the protein (44). However, Rutjes pointed out that VP22 could have a negative effect on the solubility of some fusion proteins, which consequently precluded intercellular trafficking (40).

Although it was reported that amplification of the immune response might occur via mechanisms other than VP22-mediated intercellular spread of antigen (35), VP22 could nevertheless be used to augment the potency of DNA vaccines (20, 38, 41). Moreover, it has been shown that VP22 could transport the mRNA to uninfected cells (43). All of these findings suggest that the VP22-mediated protein delivery system can be a useful and potential tool to enhance the efficacy of gene therapy.

PROMOTION OF PROTEIN SYNTHESIS AND ACCUMULATION OF mRNAs

Previous studies of a UL49 deletion mutant showed that VP22 greatly enhanced plaque size and viral replication at low multiplicities of infection (8, 37). UL49 deletion virions also contained decreased amounts of ICP0, gE and gD (8, 15). A recent investigation,

aiming to determine whether the latter phenomenon was due to decreased virion incorporation or decreased synthesis of ICP0, gE and gD in the absence of VP22, showed that VP22 was required for optimal protein synthesis at late times in infection and the accumulation of at least gE, gD and virion host shutoff (vhs) mRNAs at early times in infection (7). Interestingly, VP22 effects on protein synthesis and mRNA levels are distinct and separable with regards to both timing during infection and the genes affected (7). VP22 has also been shown to interact with mRNA and is found in both the nucleus and cytoplasm pointing to a possible direct role for VP22 in mRNA export and/or translation. VP22 interacts with tegument protein VP16, a component of primary enveloped virions, which is also a potent transcriptional activator of viral immediate-early (IE) genes and can recruit various transcriptional coactivators to target gene promoters (13). In addition, the VP16/VP22 complex can interact with the UL41-encoded protein vhs which is a ribonuclease that specifically cleaves host and viral mRNAs (48). So VP22 may promote protein synthesis and contribute to mRNA accumulation by enhancing the effect of VP16 or modulating the RNase activity of vhs. The details of the mechanisms involved in protein synthesis and accumulation of mRNAs require further investigation.

POSSIBLE TRANSCRIPTIONAL REGULATION FUNCTION

VP22 interacts with VP16 and enhances the effect of VP16 (13). VP22 locates to the cell nucleus where it binds to chromatin (1, 28) and it also specifically inhibits nucleosome assembly by binding to TAF-1 (50). In addition, VP22 has a special relationship with

the important viral transcriptional regulatory factor ICP0 (15). Previous studies on VP22 indicated that it was highly phosphorylated during HSV-1 infection (36), it is correlated in its function to certain links during virion assembly (8, 15), and has the capacity to stabilize the microtubule (10, 21). All these studies showed that VP22 might have some transcriptional regulation function. A recent paper demonstrated that VP22 alone in the cellular environment inhibited transcriptional activation of different viral transcriptional regulatory factors on different viral gene promoters (55). Perhaps VP22 performs these functions by interacting with viral or cellular proteins. However whether VP22 has the same transcriptional regulation function during virus infection is still unknown.

ITS INTERACTION PROTEINS AND POTENTIAL FUNCTIONS

The exact roles of VP22 in the virus life cycle remain poorly understood. However, it is likely that various dynamic interactions of VP22 with different cellular proteins and/or viral proteins are required.

VP22 exhibits the properties of a classical microtubule-associated protein, reorganizing and stabilizing the host cell microtubule network and hyperacetylating these stabilized microtubules (MTs) (10, 21). While the role of the VP22-microtubule interaction during infection has yet to be determined, it would seem that phosphorylation may play an important role in ensuring that this interaction is not dominant over the other activities of VP22 during infection (39) and microtubule reorganization may function as a mechanism to regulate VP22 nuclear localization (21). Microtubule reorganization is important for viral exocytosis and for transport of HSV-1 capsids to the

nucleus (17). In addition, VP22 interacts with non-muscle myosin IIA (NMIIA) (49), a motor protein which is recruited to the actin cytoskeleton and has been reported to be involved in numerous dynamic cellular processes, including Golgi budding, vesicle secretion, cell spreading, cleavage and migration, and cell adhesion (45). The VP22-myosin interaction provides evidence for an interaction with a network of myosin-actin filaments other than the microtubule/dynein system. NMIIA may be involved in virus maturation (49). The interaction between VP22 and MT/NMIIA could be relevant to assembly and egress, and could play a role in virus transport. Further work will be necessary to identify the exact role of the interactions.

A previous study showed that VP22 interacted with a cellular histone chaperone TAF-I (50), which has been shown to promote more ordered transfer of histones to naked DNA through a direct interaction with histones (31). TAF-I, as a subunit of the INHAT (inhibitor of acetyltransferases) protein complex, also binds to histones and masks them from acting as substrates for the acetyltransferases p300 and PCAF (46). The study further showed that interaction with VP22 inhibited the activity of TAF-I α in chromatin assembly, while, conversely, overexpression of TAF-I α suppressed HSV infection *in vivo*. VP22-binding proteins may form an INHAT complex pp32/PHAPI that could be relevant to HSV DNA organization early in infection (50). But other functions such as transcriptional regulation cannot be excluded and further work is necessary to prove this interaction physiologically.

VP22 interacts with membranes and is also believed to possess the ability to multimerize, and more

specifically with membranes of the acidic compartments of cells that may include the *trans*-Golgi network (TGN) (6, 33). A previous study of VP22, which used live-cell fluorescence during virus infection, demonstrated a punctate cytoplasmic localization reminiscent of the Golgi apparatus (12). Current evidence supports a model that final tegumentation and envelopment of herpesviruses occur when nucleocapsids bud into cytoplasmic vesicles, which may be derived from the TGN (6). Consistent with this observation, VP22 is packaged into virions during final envelopment as nucleocapsids bud into TGN-derived vesicles (30). VP22 can interact with the cytoplasmic tails of gD and gE, and may facilitate the interaction of viral nucleocapsids with glycoproteins lining up on the membranes of TGN-derived vesicles, perhaps also through its interaction with VP16 (62). The interaction between VP22 and VP16, gD or gE/gI may promote secondary envelopment.

A novel interaction between VP22 and the US9 protein (pUS9) has been screened by yeast two-hybrid recently (23). pUS9 of HSV-1 is a tegument protein and the US9 gene is highly conserved among the members of the alpha subfamily of herpesviruses (5). More recent studies indicated that pUS9 is associated with the endoplasmic reticulum and Golgi apparatus, as well as with cytoplasmic, unenveloped capsids. On the other hand, a detailed study of the pseudorabies virus (PRV) demonstrated that pUS9 of PRV was a type II membrane protein associated with Golgi or perinuclear membranes (22). Although the US9 gene is dispensable for viral replication in cultured cells, it is believed that pUS9 is a lysineless ubiquitinated protein that interacts with the ubiquitin dependent pathway for degradation of proteins. This function

may be initiated at the time of entry of the virus into the cell (5). A recent observation showed that pUS9 was necessary for long distance anterograde axonal transport of viral nucleocapsids (22). It could represent an additional viral mechanism to regulate specific cellular and virus functions. This interaction is likely to be involved in the process of secondary envelopment during viral assembly. However, whether this is a structural or regulatory interaction requires further investigation.

pUL46 (VP11/12) is another novel protein which interacts with VP22 (23), it is an abundant virion tegument phosphoprotein with approximately 1200 molecules per virion (18). pUL46 and VP22 have similar localization and both are membrane-associated proteins, however, pUL46 binds the membrane less strongly than VP22 (34). It has been reported that the HSV-1 DNA fragment containing the UL46 gene enhanced the efficiency of VP16-mediated gene expression (56). Also pertinent to this report is the observation that VP22 interacts with VP16 (13); it was reported that pUL46 could also interact with VP16 (51). The interactions among these three tegument proteins indicate that they may simultaneously play some role in the replication of the virus. It seems that pUL46 is an inner layer of the tegument protein which associates directly with capsids, while VP16 and VP22 belong to the peripheral layer composed of proteins that interact with the envelope. The mechanism of interaction between VP22 and pUL46 still needs further study.

CONCLUSION

Herpesviruses are highly disseminated in nature. HSV-1 is one of the most intensively investigated

herpesviruses which serves as a model and a tool for a wide range of applications including the study of translocation of proteins, membrane structure, gene regulation, gene therapy and cancer therapy. VP22, the essential tegument protein of HSV-1, is conserved within the alpha-herpesvirus family, and has multiple functions in virus replication and spread.

VP22 has intercellular trafficking properties. It is necessary for localization of ICP0 to putative sites of viral assembly within the cytoplasm and can also package RNA into the virion. Recently, many novel functions attributed to the HSV-1 VP22 protein were shown, including promotion of protein synthesis at late times in infection, accumulation of a subset of viral mRNAs at early times in infection and a possible transcriptional inhibition function in some genes expression.

VP22 can accumulate in the nucleus as well as exploit the host cytoskeleton suggesting that this unusual tegument protein may have an important role in herpesvirus infection, replication and pathogenesis. Although the exact molecular mechanisms are still not clearly understood, exploring the mechanism of how VP22 is imported to the nucleus may advance the knowledge of the pathogenesis of the herpesviruses and enable the development of new antiviral approaches.

VP22 can interact with at least five virus proteins (VP16, gE, gD, pUS9 and pUL46) and at least three cellular proteins (TAF-I, NMIIA and MT), and plays different roles according to these different interactions. From these reports we can conclude that the interactions of VP22 with different cellular proteins and/or virus proteins are important for its functionality. So it is necessary to establish the network of protein (VP22)-protein (host/virus) interactions that facilitate

further definition of the potential roles and possible mechanism of VP22. Understanding the mechanism of the multifunctional regulatory protein VP22 may advance our knowledge of the HSV-1 and promote the exploitation of efficient methods for preventing viral infection and antiviral therapy.

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