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Varicella-Zoster Virus Open Reading Frame 66 Protein Kinase and Its Relationship to Alphaherpesvirus US3 Kinases

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Abstract

The varicella-zoster virus (VZV) open reading frame (ORF) 66 encodes a basophilic kinase orthologous to the US3 protein kinases found in all alphaherpesviruses. This review summarizes current information on the ORF66 kinase, and outlines apparent differences from other US3 kinases, as well as some of the conserved functions. One critical difference is the VZV ORF66 kinase targeting of the major regulatory VZV IE62 protein to control its nuclear import and assembly into the VZV virion, which is so far unprecedented in the alphaherpesviruses. However, ORF66 targets some cellular targets which are also targeted by US3 kinases of other herpesviruses, including the histone deacetylase-1 and 2 proteins, pathways that lead to changes in actin dynamics, and the targeting of substrates of protein kinase A, including the nuclear matrix protein matrin 3.

1 Introduction

The open reading frame (ORF) 66 protein kinase is one of two varicella-zoster virus (VZV) protein kinases initially identified based on genomic position and homology to herpes simplex virus (HSV) kinases and the presence of classical structural motifs found common to all ser/thr kinases. Homologs of ORF66 are often termed the US3 kinases, since they are found in the unique short region of the genome of all sequenced neurotrophic alphaherpesviruses (and probably all alphaherpesviruses), but are absent in members of beta and gamma herpesviruses. By influencing phosphorylation states – the key means of reversible protein functional modulation – they affect many events in infection. Members of the family as a whole influence processes such as survival of the infected cell to apoptosis, the state of permissivity to gene expression, avoidance of immunity, modulating cellular pathways affecting host actin dynamics, and influencing the nuclear structure and nuclear membrane to enable assembly of virus components. The ORF66 kinase is clearly important

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for VZV growth in certain cell types relevant to human disease. Thus, interest in the ORF66 kinase and the search for its targets continues.

2 Genetics

VZV ORF66 lies in the unique short region of the VZV genome (nucleotides 113,037–114218 in VZV Dumas, 113142–114323 in POka). Its genetic disruption in VZV, first reported by Heineman et al. 1996, established it as not required for growth in cell cultures used for VZV propagation. In this regard, ORF66 mirrors similar US3 mutants of HSV, pseudorabiesvirus (PRV), and marek's disease virus (MDV). In the vaccine Oka background, ORF66 disruption had no effect on viral growth rates, but in the parent Oka VZV background, disruption caused 3–20 fold drop in peak growth levels compared to parental virus, depending on host cell type. As the γI gene lies immediately downstream of ORF66, the complete ORF66 gene cannot be deleted entirely without affecting γI expression, as ORF66 contains control elements in the γI promoter.

VZV mutants lacking ORF66 kinase activity do show more impaired growth in certain cell types or in organ culture models, suggesting that the host cell dictates the importance of the kinase to infection. VZV lacking ORF66 grows poorly in cultured T cells (Soong et al. 2000) and in human thymus/liver xenografts in severe combined immunodeficiency (SCID-hu) mice (Moffat et al. 1998; Schaap-Nutt et al. 2006; Schaap et al. 2005). This has relevance to human disease, since current models of VZV infection propose that T cells transport VZV from the tonsillar respiratory epithelium to the skin (Ku et al. 2005). In T cells, VZV lacking ORF66 kinase shows greater sensitivity to IFN-γ treatment, and increased levels of apoptosis. Electron microscopic examination of such cells reveals an apparent defect in the formation of nucleocapsids, but do not show the accumulation of abundant nucleocapsids at imaginations of the inner nuclear membrane, as seen for US3 kinase-deficient HSV-1 and PRV (see Sect. 5.3). The molecular basis for the VZV phenotypes is not yet understood.

VZV without functional ORF66 also replicates poorly in primary corneal fibroblasts obtained from human corneal stroma donor rims (Erazo et al. 2008). This is significant to human disease, as the cornea is often infected during zoster reactivating from the fifth cranial nerve. Corneal fibroblasts were initially evaluated to investigate possible roles of the ORF66 kinase on actin dynamics, as these cells develop prominent stress fibers when cultured on plastic support. Using recombinant VZV in which GFP was tagged N-terminally to ORF66 kinase, a truncated form or a kinase-inactive form, it was found that VZV without kinase was blocked for replication at a stage following the initial round of replication after infection with infected human MRC-5 cells. VZV lacking ORF66 formed microfoci of GFP positive cells that subsequently fail to expand over time. The basis for growth impairment is not yet known, but data suggested it was not a result of differential regulation of apoptosis or regulation of cellular localization of IE62 (Erazo et al. 2008).

3 ORF66 Structure and Characteristics

The ORF66 protein kinase, at 393 residues, has a predicted weight of 44 kDa. It is a phosphoprotein (Stevenson et al. 1994) that migrates in our hands at 55 kDa as two forms that appear to be differentially phosphorylated. There is no evidence to suggest alternative forms initiating at alternative ATG residues, as seen for US3 kinases of HSV and PRV. ORF66 has a conserved 285 residue kinase (catalytic) domain spanning amino acids 93–378 that has homology to all ser/thr kinases, which consists of 12 subdomains that fold into a characteristic 3-dimensional active core structure to transfer a γ-phosphate from ATP to the hydroxyl group of a specific S/T residue within its protein substrate (see Fig. 1). These subdomains are remarkably invariant within the eukaryotic protein kinase superfamily

(Hanks and Hunter 1995). Conservative mutations (D206E and/or K208R) made in the central catalytic domain spanning residues 203–211 disrupt kinase activity. Comparing ORF66 to the cellular ser/thr kinases predicts the ATP binding residue is likely K122, and a K122A mutation also abrogates kinase activity. Using the entire gene in blast searches, the closest cellular homologs are human serine/threonine kinase 9 (also known as cyclin dependent kinase – like 5) and the yeast cell cycle regulator cdc28 (McGeoch and Davison 1986; Schaap et al. 2005). However, the amino terminal region of the protein has a high ratio of acidic residues, as found in all US3 kinases and also in the p21-activated kinases upstream of Cdc42/Rac pathways (see starred residues in Fig. 1). The precise role of the acidic domain is not clear.

A significant fraction of ORF66 kinase is insoluble in most buffers designed to solubilize the protein without disturbing its kinase activity. ORF66 solubility is increased in higher pH buffers, as found for HSV-1 US3 kinase, and our optimal buffer used to solubilize GST-tagged ORF66 from baculovirus-infected cells contains 20 mM Tris-HCl pH8.5, 50 mM KCl, 1 mM EDTA, 1 mM DTT, 1%NP40, and 0.5% DOC. Kinase activity **in vitro** is optimal in 20 mM Hepes pH 7.5, 50 mM Mn^{2+}, and 50 mM KCl. ORF66 is not inhibited by 10 μg/ml heparin (which effectively blocks casein kinase II activity), so this is included in assays. Levels of 10 mM Mg^{2+} can also be used as the cation in the ORF66 **in vitro** kinase assay (Eisfeld et al. 2006).

The cellular localization of ORF66 protein has an unusual distribution. While initial studies using ORF66 specific antibodies first indicated ORF66 as a cytoplasmic protein (Stevenson et al. 1994), studies from our lab using epitope tagged or functional GFP-ORF66 fusions indicate both nuclear and cytoplasmic distribution in VZV-infected cells and in cells expressing the kinase autonomously, with nuclear forms predominating (Eisfeld et al. 2007; Kinchington et al. 2000; Schaap-Nutt et al. 2006). Nuclear ORF66 shows a discrete and distinct punctate nuclear accumulation, forming rings of puncta surrounding the infected cell nucleolus (Eisfeld et al. 2007; Kinchington et al. 2000). Characterization of these ORF66 speckles is in progress, and these appear dynamic (Eisfeld and Kinchington, manuscript in preparation). Functional ORF66 also associates with replication compartments early in infection in MRC-5 cells, whereas kinase dead (kd) forms (D206E, K208R) accumulate in both nuclear replication compartments and nuclear rim of late stage VZV-infected cells, colocalizing with major capsid protein (MCP) (Eisfeld and Kinchington, manuscript in preparation). This suggests that ORF66 kinase activity influences its own cellular distribution, and may be associated with capsid assembly and/or egress.

4 ORF66 Targets

Only one target of ORF66, the IE62 regulatory protein, has been extensively characterized at the time of this review (Table 1). The two sites targeted strongly suggest ORF66 is a basophilic kinase that phosphorylates ser/thr residues preceded by multiple arginine or lysine residues, particularly at −2 and −3 positions. This is consistent with target motifs of PRV and HSV US3 kinases determined by **in vitro** peptide substrates, with an optimal motif of $(R)_{\mathbf{n}}$ **X**(S/T)−**YY** (where **n** is >2. S/T is the target site where either serine or threonine is phosphorylated, **X** can be absent or any amino acid but preferably Arg, Ala, Val, Pro, or Ser, and **Y** is similar to **X** except that it cannot be an absent amino acid, prolinc, or an acidic residue) (Benetti and Roizman 2004). However, studies on the US3 kinase suggest the optimal motif is overly restrictive, and sites of phosphorylation with much lower matches to the consensus have been reported on lamin C (Mou et al, 2007). Of particular note is that both VZV ORF66 and HSV-1 US3 kinase target motifs overlap that targeted by-Protein kinase A (PKA). Using antibodies to the phosphorylated serine in the PKA target motif, novel substrates are detected in extracts of VZV-infected cells that are not found in VZV

kinase-deficient infected cells, suggesting the kinase targets multiple cellular proteins or induces activation of cellular kinases that target phospho-PKA motifs. Interestingly, the antibody identifies radically different protein profiles in the same cell type infected with VZV, HSV, and PRV (Erazo et al. manuscript in preparation).

4.1 Autophosphorylation

Protein kinase autophosphorylation is frequently employed to uphold the specificity of kinase functions (Wang and Wu 2002); thus it is not surprising that ORF66 autophosphorylates. Disruption of the kinase catalytic domain or the ATP binding residue results in a poor ^{32}P-incorporation into the protein within VZV-infected cells and in **in vitro** reactions with purified kinase. The sites of phosphorylation remain to be determined (Eisfeld et al. 2006; Kinchington et al. 2000). Assuming kinase targeting of serines is preceded by basophilic residues, likely candidate sites are located at $KRS_{331}SRK$ and $RHRPS_{368}$. However, mutagenesis studies indicate that the S_{331} residue is not required for kinase activity (Schaap et al. 2005). VZV ORF66 has no obvious equivalent to the S147 autophosphorylation residue found to be the site of HSV-1 US3 autophosphorylation (Kato et al. 2008), and it is not yet known if ORF66 is phosphorylated by ORF47. The US3 kinase is phosphorylated by the UL13 kinase (Kato et al. 2006) in HSV-1 infected cells.

4.2 IE62

ORF66 kinase targets IE62, the major regulatory protein of VZV. IE62 is a nuclear transcriptional regulatory protein that drives VZV transcription by interacting with transcriptional activators, components of the mediator complex, and members of the general factors involved in recruitment of RNA pol II complex (Ruyechan et al. 2003; Yang et al. 2008). While the underlying mechanisms by which IE62 acts are not resolved, its ability to partly substitute for HSV ICP4 infers that both IE62 and ICP4 act in a similar manner. The targeting of IE62 by the VZV ORF66 kinase was serendipitously discovered in studies to examine the influence of ORF47 kinase on IE62 functions, as IE62 is an ORF47 kinase target (Ng et al. 1994). Cells transfected to express IE62 with or without the ORF47 kinase showed IE62 as a predominantly nuclear protein. The IE62 nuclear localization signal is a classical SV40-like signal high in arg/lys rich residues mapping to residues 677–85 (Kinchington and Turse 1998). However, IE62 coexpressed with the ORF66 kinase showed accumulation of abundant cytoplasmic forms of IE62, mirroring that seen in late stage VZV-infected cells. While IE62 is nuclear early in VZV infection before ORF66 is expressed, IE62 levels build in the cytoplasmic compartment as ORF66 accumulates, until some infected cell nuclei appear devoid of IE62. Cytoplasmic IE62 does not form in cells infected with VZV lacking functional ORF66 kinase, establishing that kinase activity is required. The sites of phosphorylation on IE62, mapped using plasmids expressing IE62 peptides in ORF66 transfected and VZV-infected cells, are predominantly restricted to IE62 residues S686 and S722. ORF66 directly phosphorylated 1E62 **in vitro**, and bacterially expressed IE62 peptides with both or one serine intact remained a target for purified VZV ORF66 kinase **in vitro**, whereas loss of S686 and S722 abrogated the ability of IE62 to be an ORF66 target.

The ORF66 kinase-mediated regulation of IE62 has not been reported for corresponding proteins of other alphaherpesviruses, but it reflects the regulated nuclear import of many cellular proteins through phosphorylation (Harreman et al. 2004; Jans and Hubner 1996). As phosphorylation is reversible, it can enable multifunctional proteins to be controlled by their relocation to different cellular compartments. The nuclear exclusion of IE62 in VZV infection enables the packaging of abundant levels of IE62 into VZV virions, at about 5% of the level of the major capsid protein. Virions obtained from VZV-infected cells lacking kinase show virtually no structural forms of IE62 (Kinchington et al. 2001). It was

concluded that nuclear exclusion of IE62 allows it to relocate to the **trans**-Golgi network, where VZV tegument is added to the egressing nucleocapsid (Kinchington et al. 2001). Virion packaging of IE62 may allow the introduction of preformed IE62 into the newly infected cell to promote the first events of infection, although this remains to be formally shown. The importance of the targeting of S686 in the ORF66 driven relocation of IE62 was shown using a VZV recombinant containing S686A changes in both copies of IE62 in the VZV genome. Such virus expressed IE62 protein which did not relocate to cytoplasm or become packaged during infection, despite the presence of a functional ORF66 kinase (Erazo et al. 2008).

We postulate that this interaction may come to play during VZV latency. VZV infects sensory nerve endings during varicella and establishes latency in neural nuclei in dorsal root ganglia. In contrast to HSV-1, where there is predominant silencing of protein expression and expression of non-coding latency associated RNA transcripts, VZV latency is characterized by expression of several lytic mRNAs and some regulatory proteins which show nuclear exclusion. Transcripts and proteins of ORF62 and the ORF66 kinase have been reported in human latently infected tissue (Cohrs and Gilden 2007; Cohrs et al. 2003), and IE62 shows predominantly cytoplasmic distribution (Cohrs et al. 2003; Lungu et al. 1998). It has been proposed that VZV latency is maintained by preventing nuclear functions of the regulatory proteins through nuclear exclusion. Our discovery may mechanistically explain IE62 nuclear exclusion during latency.

Interestingly S686 in IE62, which immediately follows the nuclear import signal, is highly conserved in virtually all the alphaherpesvirus ICP4/IE62 homologs. This suggests that their cellular localization may also be regulated by phosphorylation. Indeed, cotransfection studies suggest US3 kinases reduce nuclear import of the corresponding IE62/ICP4 homolog (Yee and Kinchington, unpublished data). However, HSV-1 ICP4 cytoplasmic forms are more reliant upon the functionality of the HSV ICP27 protein (Sedlackova and Rice 2008).

4.3 Matrin 3

The ORF66 kinase targeted sites on IE62 suggest the motif targeted by ORF66 overlaps that of PKA. Roizman and colleagues addressed novel targets of the US3 kinase by probing cell extracts with antibodies directed to the PKA phosphorylated substrates. While Desloges et al. suggested VZV modulated PKA activity (Desloges et al. 2008), we will shortly report (Erazo, Yee and Kinchington, manuscript in preparation) that VZV generates a profile of substrates that includes a prominent species of 125 kDa that was not detected in uninfected cells or in cells infected with VZV deficient in ORF66 kinase activity. Furthermore, this 125 kDa species was seen in cells autonomously expressing functional ORF66 kinase, but not the kinase-inactive form. Thus, this reagent identified a cellular protein significantly phosphorylated, directly or indirectly, by the ORF66 kinase. LC MS/MS analyses of immunoprecipitates with the PKA phospho-specific antibody show the 125 kDa species is matrin 3. Using antibodies to matrin 3 in conjunction with the PKA phospho-substrate antibodies, Matrin 3 phosphorylation only occurred in cells expressing ORF66 by adenovirus mediated transduction or by VZV infection, but not in the same cells if the expressed ORF66 kinase is disrupted or inactivated.

The consequences of matrin 3 phosphorylation to infection are not yet clear. Matrin 3 is one of the 12 major nuclear matrix proteins, but remains only scantily studied. One prior report detailing matrin 3 phosphorylation suggested rapid degradation of matrin 3 following PKA-mediated phosphorylation, induced by NMDA receptor activation of cultured neurons (Giordano et al. 2005). This degradation eventually led to cell death. VZV ORF66 may drive matrin 3 degradation to disrupt nuclear structure to promote nucleocapsid assembly, as suggested for nuclear matrix in HSV-1 capsid assembly (Bibor-Hardy et al. 1985). Matrin 3

also has structural features homologous to RNA binding motifs, and is involved in retaining hyper-edited RNAs and double-stranded RNAs in the nucleus that arise through errant processing (Zhang and Carmichael 2001). Matrin 3 acts as a gatekeeper of such RNAs to prevent their erroneous translation. Matrin 3 also interacts with hnRNP-L involved in regulating RNA splicing (Zeitz et al. 2009). Thus its phosphorylation by ORF66 may influence RNA processing in VZV-infected cells. Matrin 3 is also phosphorylated by the US3 kinases of PRV and HSV-1, despite the different profiles of proteins recognized by the anti-phospho-PKA-substrate antibody (Erazo, Yee and Kinchington, manuscript in preparation).

4.4 Histone Deacetylases

Transcription is strongly influenced by the chromatin state of the template DNA that, in turn, is under an elaborate control system that modulates histone binding and condensation. A key component is the reversible post translational modification of histones through the addition and subtraction of acetyl groups to their lysine tails. In general, permissive gene expression is promoted by histone acetyl transferases which acetylate histones to relax DNA binding and condensation. Silencing of expression is partly driven by their deacetylation, mediated by histone deacetylases (HDACs). HDACs are an ancient family of enzymes that have a major role in numerous biological processes. Eleven different HDAC isoforms have been identified in mammalian genomes and these are classified into four different families: class I (HDAC1, 2, 3, and 8), class II, (HDAC4, 5, 6, 7, 9, and 10), sirtuin class III and class IV (HDACII) (Haberland et al. 2009; Schwer and Verdin 2008). Because HDACs lack intrinsic DNA-binding activity, they are recruited to target genes through direct association with transcription regulatory proteins. HDAC activity is controlled by phosphorylation by numerous cellular kinases (Pflum et al. 2001). This blocks their deacetylase functions and promotes a cellular permissive state of transcription. The herpesviral infected cell is favored by a pro-active transcriptional state in which deacetylation is inhibited. In HSV-1 infected cells, multiple mechanisms are involved in the inactivation of HDAC activity, including HSV-1 ICP0, which dislodges the LSD1/CoREST/REST complex from HDAC1 and HDAC2. disrupting the silencing effects of this repressor complex on viral promoters. More recently, it was reported that HDAC-1 and 2 showed novel forms which were induced by the Us3 kinase, and that cells expressing the US3 kinase showed a more pro-active transcriptonal state.

Recent work suggests that HDAC-1 and 2 are also modulated in VZV-infected cells in an ORF66-dependent manner (Walters et al. 2009). HDAC-1 and 2 show novel slower mobility forms in SDS-PAGE gels of VZV-infected cell extracts that are not apparent if the ORF66 kinase is deleted. The slower form is differentially phosphorylated and is also seen in cells expressing the ORF66 protein kinase autonomously by transaction or by transduction with ORF66 expressing adenoviruses. Mapping of the sites of phosphorylation show it occurs at a specific residue in the C terminal domain of both proteins which are preceded by basic residues at −2 and −3 positions, consistent with PKA target motifs.

Functional consequences of ORF66 activity affecting HDACs has been suggested from studies using the HDAC inhibitors sodium butyrate. At 1 mM, this inhibitor relieves some of the attenuation of the ORF66 negative VZV as compared to the parental virus. Thus it seems that a prime function of the kinase is to regulate cell permissivity at the transcriptional level through interactions with HDAC-1 and 2 and possibly other HDACs.

5 Cellular and Viral Activities Modulated by the ORF66 Protein Kinase

5.1 MHC-I Surface Presentation

The ORF66 protein kinase mediates VZV-encoded immune evasion strategies. In the host, viral and cellular antigenic peptides are presented on the cell surface for CD8+ T cell recognition in conjunction with the major histocompatibility complex type I or MHC-I. Most herpesviruses have mechanisms to reduce surface presentation of MHC-I coupled viral antigens, presumably to allow prolonged survival of the cell in the presence of a developed immune system. In MHC biogenesis, antigenic peptides generated by the host 26S proteasome are actively transported to the ER lumen by the Transporter of Antigen Presentation (TAP), composed of a heterodimer of TAP-1 and TAP-2. TAP is inhibited by many viruses, because its inhibition affects MHC-I A and B, the main antigen presenters, but not MHC-I types that are needed to signal to natural killer cells. TAP is blocked in HSV-1 infected cells by the immediate early protein ICP47, of which there is no homolog in VZV. Varicelloviruses are reported to have a second gene that blocks TAP, of which the bovine herpesvirus UL49.5 is the most well characterized (Koppers-Lalic et al. 2008). However, we and others have not seen evidence that VZV ORF 9.5 has similar activities (Eisfeld et al. 2007). In the ER lumen, MHC-I heavy chain (Hc) bound to beta 2-microglobulin is stabilized by several chaperones (tapasin, ERp57 and calreticulin) until it couples with TAP and the antigenic peptide. The peptide loading complex can be disrupted or actively inhibited by some viral MHC-I modulators (e.g., human Cytomaglovirus US2). Once loaded, the antigenic peptide is processed to high affinity forms, which mature through the secretory pathway via the Golgi to the cell surface. **Cis-to medial**-Golgi transport is concurrent with conversion of high mannose glycan side chains to complex endoglycosidase-H (endo H) resistant forms.

It is not surprising that VZV downmodulates surface antigen presentation (Cohen 1998; Abendroth et al. 2001), as VZV has lymphotropic parameter in its human pathogenesis, and can sustain infection in multiple cell types over a prolonged period, including professional antigen presenting cells and chronic antigen-expressing neurons during latency. The lack of an ICP47 homolog suggests VZV uses novel mechanisms to mediate this block. Following an initial report by Abendroth et al. in which reduced MHC-I expression in ORF66 expressing cells was observed, we reported that surface MHC-I was reduced in ORF66 expressing cells mediated by transfection, adenovirus mediated transduction, or in recombinant viruses expressing GFP tagged forms of ORF66 but was not downregulated to the same extent in the corresponding conditions when the expressed kinase was disrupted or abrogated (Eisfeld et al. 2007). In both adenovirus transduced and VZV-infected cells, the ORF66 kinase induces the accumulation of endoglycosidase H sensitive MHC-1 forms, suggesting a block either at the assembly stage or the Golgi maturation step prior to **cis to medial** Golgi processing. In VZV infections without ORF66 kinase, MHC-1 processing is still partly blocked as compared to control cells, suggesting that additional mechanisms exist for VZV to block surface MHC-I (Eisfeld et al. 2007). In this respect, VZV is like many herpesviruses, and employs overlapping mechanisms. This is currently under further study.

5.2 IFN Signaling

Schaap et al. demonstrated that expression of ORF66 correlated with a differential level of signaling following IFNγ treatment of VZV-infected T cells. Specifically, the formation of phospho-Stat in T cells following IFNγ binding to its receptor was significantly diminished with ORF66 expression as compared to VZV infections lacking functional ORF66 (Schaap et al. 2005). It is not yet resolved as to how ORF66 blocks this activity, but it is notable that Roizman and colleagues have recently indicated that the HSV-1 US3 kinase may phosphorylate IFN-γ**Ra** to prevent its signaling (Liang and Roizman 2008).

5.3 Apoptosis

Arvin and colleagues also demonstrated that ORF66 protein kinase modulates the apoptosis of T cells that have been proposed to mediate dissemination of VZV from respiratory sites of infection to skin (Ku et al. 2005). VZV lacking ORF66 grew to levels 2 logs lower than parental virus in cultured human T cells but not in MeWo cells and the expression of the kinase conferred marginal growth advantage in skin xenografts (Schaap et al. 2005). T cells infected with a kinase-inactive G102A mutant showed increased levels of active caspase 3, the executioner protease in apoptosis, suggesting that loss of the ORF66 kinase correlated with VZV inability to check the development of apoptosis from VZV infection in this cell type (Schaap et al. 2005). These findings imply that ORF66 has an important function in extending the survival of infected T cells until they are able to home in on the skin (Schaap-Nutt et al. 2006). Therefore, inhibition of apoptosis may be the contributing function or the defining function of ORF66 needed for VZV propagation in T cells. It has also been reported that VZV modulates the PDK/Akt pathway, involved in regulation of apoptosis. Expression of ORF66 transiently or by VZV-infected MeWo cells is involved in pro-survival signaling by activation of Akt, indicated by the increase of Akt phosphorylation at serine 473, that decreased when 66 was not expressed (Rahaus et al. 2007). This may also partly explain the growth deficit of VZV in this cell type (Moffat et al. 1998). However results from studies of ORF66 kinase deficient infections in human corneal fibroblasts, which are very restrictive for such mutants, indicated no significantly increased levels of apoptosis. Recent work has revealed that the role of apoptosis in HSV-l infection is more important in highly replicating or transformed cells than in primary cell lines (Nguyen et al. 2007).

6 Alphaherpesvirus US3 Kinase Studies that Guide the Search for Roles of ORF66

Several roles of ORF66 may be speculated from identified roles of the HSV and PRV US3 kinases, since there are clear structural similarities. In addition to the kinase catalytic domain, all have a high proportion of acid residues in the amino terminal region, although this is functionally ill-defined. Thus we summarize the known features of the US3 kinases of other alphaherpesviruses.

6.1 US3 Kinases and Inhibition of Apoptosis

Viral perturbation of the host cell often triggers apoptosis, and herpesviruses have mechanisms to block programmed cell death and thus extend cell survival time to allow for viral replication (Aubert and Blaho 2001). The ability of the HSV-1 US3 kinase to block apoptosis has been the most extensively examined, although the US3 kinases of VZV, HSV-2, and PRV may have similar activities. US3 kinases block apoptosis induced by the virus as well as by a variety of ectopic treatments. HSV-1 US3 is one of at least four blockers of apoptosis (in addition to UL39, glycoproteins gD, gJ). Kinase activity is required in HSV and PRV, suggesting that cellular components are phosphorylated. The possible targets include the pro-apoptotic protein Bad, whose phosphorylation (and inactivation) was found to be US3-dependent (Cartier et al. 2003b). Recent studies indicate HSV-1 US3 kinase acts at a post-mitochondrial level, since US3, but not its inactive form US3 K220N, inhibited the cleavage and activation of procaspase 3, the zymogen form of caspasc 3 – a major effector of the pro-apoptotic pathway. This was also phosphorylated **in Vitro** by US3 (Benetti and Roizman 2007).

Two forms of HSV-1 US3 have been seen initiating at different ATGs and these appear to differ in their ability to block apotosis. A US3 blocked apoptosis but US3.5 did not, even though both were able to localize in mitochondria (Poon et al. 2006a). PRV also encodes

two forms, a long (US3a) and short isoform (US3b) differing by an additional N terminal 54 residues from US3a that encodes a mitochondrial localization signal (Calton et al. 2004). However both block apoptosis, suggesting that the mitochondrial signal and localization of the protein may only be partly responsible for the increase in its anti-apoptotic function (Geenen et al. 2005). There is evidence for the block in apoptosis to be downstream of cell signaling pathways activated by US3. HSV-1 US3 activates PKA, and PKA activation by forskolin inhibits apoptosis (Benetti and Roizman 2004). In sum, evidence points to the US3 kinases as one means to prevent programmed cell death, but several cellular targets may be involved.

6.2 US3 Modulation of HDAC

As just detailed, inhibition of HDACS is needed for efficient viral gene expression (Hobbs and DeLuca 1999; Poon et al. 2003). Although ICPO is thought to be the key player in blocking genomic silencing in HSV-1, US3 may contribute by post translational modification of HDAC1 and HDAC2 (Poon et al. 2003; Poon and Roizman 2007). HSV-1 US3.5 shares this ability (Poon et al. 2006a). US3 and 3.5 enable viral or host gene expression from restrictive cells and enhance expression from permissive cells transduced with baculovirus carrying CMV immediate early promoter-driven genes (Poon et al. 2006b). While phosphorylation regulates HDAC1 and 2 enzymatic activity, it is not yet clear how HSV US3 or VZV ORF66 facilitates viral gene expression, though the suspicion is that HDAC1 and 2 are direct phosphorylation targets of US3 in infection (Poon and Roizman 2007).

6.3 Nucleocapsid Egress

Herpesvirus nucleocapsids assemble in the nucleus, and DNA packaged nucleocapsids pass through the inner (INM) and outer nuclear (ONM) membranes to the cytoplasm in an envelopment/de-envelopment fusion mechanism. Capsids then acquire most of the tegument and their final envelope as they bud through membranes of the **trans**-Golgi network (Gershon et al. 1994). In both HSV and PRV infection, deletion of the US3 kinase only moderately affects growth rates and infectious virus production in culture. However, EM analyses reveal that such mutants accumulate capsids in extended folds in between the INM and ONM, or perinuclear space (Klupp et al. 2001; Reynolds et al. 2002; Wagenaar et al. 1995). The US3 kinase thus facilitates egress of the capsid from the intranuclear space. US3 kinase mediated phosphorylation of several viral and cellular proteins have been implicated in this process, including UL34, a type 2 integral membrane protein that localizes to the INM and UL31, Both are critical regulators of primary envelopment of nucleocapsids (Kato et al. 2005; Mou et al. 2009; Purves et al. 1991; Ryckman and Roller 2004). In HSV-1 infection, the UL34 forms a complex with UL31 and displays smooth localization along the nuclear envelope, while in the absence of US3 kinase activity, the complex forms aberrant punctate accumulations at the nuclear membrane (Reynolds et al. 2001; Reynolds et al. 2002). UL34 was the first established target of the US3 kinase, but studies now indicate that its phosphorylation by US3 at the C terminal end does not appear to be directly involved (Kato et al. 2006) in directing the normal localization of the UL34-UL31 complex. The US3 kinase does phosphorylate UL31 **in vitro** (Kato et al. 2005), and prevention of phosphorylation at the serine rich N terminus of UL3l leads to virions accumulating in the perinuclear space. Thus the US3 specific phosphorylation of UL31 may facilitate virion nuclear egress (Mou et al. 2009).

Additional roles of the US1 kinase in nucleocapsid egress stem from recent studies indicating the kinase phosphorylates nuclear membrane forms of the major glycoprotein γB at the cytoplasmic tail. HSV with γB altered at the site of US3 mediated phosphoryation shows the same phenotype to the US3 kinase deletions, in that nucleocapsids accumulate in

perinuclear invaginations that protrude into the nucleoplasma. The US3 kinase may modulate γB-mediated fusion events at the ONM to allow de-envelopment during nuclear egress (Wisner et al. 2009). With regards to VZV ORF66, VZV γB does have predictable IE62-like potential motifs for phosphorylation in the cytoplasmic tail which bear similarities to that targeted by US3, but it is not yet known if ORF66 phosphorylates γB. Schaap-Nutt indicated no obvious accumulation of VZV nucleocapsids at the nuclear rim in T cells, but rather showed vastly decreased nucleocapsid formation.

The HSV US3 kinase also modulates host components of the nuclear envelope, including lamin A/C and emerin, an integral nuclear membrane protein which associates with lamin proteins (Leach et al. 2007; Mou et al. 2007). The US3 kinase leads to the redistribution of these proteins in HSV-1 infection, which normally lie just inside the nuclear membrane and act as a barrier for virions budding into the INM. In the presence of US3 kinase, emerin becomes hyperphosphorylated, increasing its mobility during infection. It was postulated that this causes dissociation of emerin and lamin A/C to facilitate the nuclear egress of nucleocapids (Leach et al. 2007). In the case of lamin A/C, it is theorized that US3 is involved in a careful balance in breaching the lamina network barrier to ease virion access to budding sites at the INM, yet maintaining laminar structure (Mou et al. 2007).

6.4 Alteration of the Host Cytoskeleton

Many viruses restructure the host cell cytoskeleton to promote viral inter and intracellular spread. The HSV-1 PRV and MDV US3 kinases have joined the group of increasing viral effector proteins reported to manipulate the host cell cytoskeleton (Smith and Enquist 2002). PRV US3 has been shown to disassemble the actin cytoskeleton and induce novel formation of actin and microtubule-containing cell projections in both the context of viral infection and in US3-transfected cells (Favoreel et al. 2005; Van Minnebruggen et al. 2003). Viral particles found within these dynamic projections were shown to move directionally towards the tip of projections as infection progressed (Favoreel et al. 2005) enabling more efficient spread to adjacent cells. This may allow more efficient infection in the presence of virus neutralizing antibodies. The US3-mediatcd actin disassembly also induced loss of cell-cell contacts and disassembly of focal adhesion which may be important to PRV spread (Van den Broeke et al. 2009a). Interestingly, US3 was found to induce activation of group A p21-activated kinases through a threonine residue in the activation loop, and phosphorylation of PAK1 and PAK2 **in vitro**. These host proteins are players in Rho GTPase signaling pathways involved in actin disassembly and lamellipodia or filopodia formation. PAK1 and PAK2 were found to be required for infection-induced actin cell projections and disassembly, respectively (Van den Broeke et al. 2009b).

In HSV-2, US3 kinase induced cell rounding and dissolution of actin stress fibers in transfected cells and US3 expressing cell lines. Dominant active forms of the RhoGTPases, Rac, and Cdc42, co-transfected with US3 kinase reduced cell rounding, suggesting that US3 affects the Cdc42/Rac signaling pathway. Interestingly, the Rho family proteins regulate various aspects of actin dynamics and can activate PAKs (Murata et al. 2000). Transient dissassembly of the actin cytoskeleton was also seen for MDV in infections and was dependent on expression of US3. Addition of cytochalasin D which inhibits G actin repolymerization, blocked MDV plaque formation. This taken together with the growth defects observed for US3-deficient MDV and the US3-dependent actin disassembly, support the idea that actin and not microtubule restructuring is important for virus intercellular spread (Schumacher et al. 2005). In contrast to PKV, MDV US3 kinase activity was not needed for actin remodeling in transfected cells (Schumacher et al. 2008), indicating US3-dependent actin disassembly may be dependent on its structure. With regard to VZV, we have found that cellular stress fibers are reduced in wild type VZV-infected cells but remain

prominent and abundant in VZV-infected cells if the kinase is disrupted. Thus, it seems likely that a common target for ORF66 and the US3 kinases is the modulation of the actin-based cytoskeleton.

7 Concluding Remarks

VZV ORF66 clearly has important multifunctional roles in the infectious processes which are highly cell type dependent. Thus it is likely that the critical functions of the kinase induce the phosphorylation of cellular targets or of cellular signaling pathways to drive the altered phosphorylation states of cellular proteins. It is clear that VZV ORF66 has functions that are specific for VZV, as well as functions that may be conserved with other alphaherpesvirus US3 kinases. What those common pathways and cellular targets are remain to be resolved.

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References

Abendroth A, Lin I, Slobedman B, Ploegh H, Arvin AM. Varicella-zoster virus retains major histocompatibility complex class I proteins in the Golgi compartment of infected cells. J Virol. 2001; 75:4878–4888. [PubMed: 11312359]

Aubert M, Blaho JA. Modulation of apoptosis during herpes simplex virus infection in human cells. Microbes Infect. 2001; 3:859–866. [PubMed: 11580981]

Benetti L, Roizman B. Herpes simplex virus protein kinase US3 activates and functionally overlaps protein kinase A to block apoptosis. Proc Natl Acad Sci USA. 2004; 101:9411–9416. [PubMed: 15192152]

Benetti L, Roizman B. In transduced cells, the US3 protein kinase of herpes simplex virus 1 precludes activation and induction of apoptosis by transfected procaspase 3. J Virol. 2007; 81:10242–10248. [PubMed: 17634220]

Bibor-Hardy V, Dagenais A, Simard R. In situ localization of the major capsid protein during lytic infection by herpes simplex virus. J Gen Virol. 1985; 66(Pt 4):897–901. [PubMed: 2984323]

Calton CM, Randall JA, Adkins MW, Banfield BW. The pseudorabies virus serine/threonine kinase Us3 contains mitochondrial, nuclear and membrane localization signals. Virus Genes. 2004; 29:131–145. [PubMed: 15215691]

Cartier A, Broberg E, Komai T, Henriksson M, Masucci MG. The herpes simplex virus-1 Us3 protein kinase blocks CD8T cell lysis by preventing the cleavage of Bid by granzyme B. Cell Death Differ. 2003a; 10:1320–1328. [PubMed: 12934063]

Cartier A, Komai T, Masucci MG. The Us3 protein kinase of herpes simplex virus 1 blocks apoptosis and induces phosporylation of the Bcl-2 family member Bad. Exp Cell Res. 2003b; 291:242–250. [PubMed: 14597423]

Cohen JI. Infection of cells with varicella-zoster virus down-regulates surface expression of class 1 major histocompatibility complex antigens. J Infect Dis. 1998; 177:1390–1393. [PubMed: 9593031]

Cohrs RJ, Gilden DH. Prevalence and abundance of latently transcribed varicella-zoster virus genes in human ganglia. J Virol. 2007; 81:2950–2956. [PubMed: 17192313]

Cohrs RJ, Gilden DH, Kinchington PR, Grinfeld E, Kennedy PG. Varicella-zoster virus gene 66 transcription and translation in latently infected human Ganglia. J Virol. 2003; 77:6660–6665. [PubMed: 12767985]

Daikoku T, Kurachi R, Tsurumi T, Nishiyama Y. Identification of a target protein of US3 protein kinase of herpes simplex virus type 2. J Gen Virol. 1994; 75(Pt 8):2065–2068. [PubMed: 8046410]

Daikoku T, Yamashita Y, Tsurumi T, Nishiyama Y. The US3 protein kinase of herpes simplex virus type 2 is associated with phosphorylation of the UL12 alkaline nuclease in vitro. Arch Virol. 1995; 140:1637–1644. [PubMed: 7487495]

Desloges N, Rahaus M, Wolff MH. The phosphorylation profile of protein kinase A substrates is modulated during Varicella-zoster virus infection. Med Microbiol Immunol. 2008; 197:353–360. [PubMed: 18066594]

Eisfeld AJ, Turse SE, Jackson SA, Lemer EC, Kinchington PR. Phosphorylation of the varicella-zoster virus (VZV) major transcriptional regulatory protein IE62 by the VZV open reading frame 66 protein kinase. J Virol. 2006; 80:1710–1723. [PubMed: 16439528]

Eisfeld AJ, Yee MB, Erazo A, Abendroth A, Kinchington PR. Downregulation of class I major histocompatibility complex surface expression by varicella-zoster virus involves open reading frame 66 protein kinase-dependent and -independent mechanisms. J Virol. 2007; 81:9034–9049. [PubMed: 17567702]

Erazo A, Yee MB, Osterrieder N, Kinchington PR. Varicella-zoster virus open reading frame 66 protein kinase is required for efficient viral growth in primary human corneal stromal fibaroblast cells. J Virol. 2008; 82:7653–7665. [PubMed: 18495764]

Favoreel HW, Van Minnebruggen G, Adriaensen D, Nauwynck HJ. Cytoskeletal rearrangements and cell extensions induced by the US3 kinase of an alphaherpesvirus are associated with enhanced spread. Proc Natl Acad Sci USA. 2005; 102:8990–8995. [PubMed: 15951429]

Geenen K, Favoreel HW, Olsen L, Enquist LW, Nauwynck HJ. The pseudorabies virus US3 protein kinase possesses anti-apoptotic activity that protects cells from apoptosis during infection and after treatment with sorbitol or staurosporine. Virology. 2005; 331:144–150. [PubMed: 15582661]

Gershon AA, Sherman DL, Zhu Z, Gabel CA, Ambron RT, Gershon MD. Intracellular transport of newly synthesized varicella-zoster virus: final envelopment in the trans-Golgi network. J Virol. 1994; 68:6372–6390. [PubMed: 8083976]

Giordano G, Sanchez-Perez AM, Montoliu C, Berezney R, Malyavantham K, Costa LG, Calvete JJ, Felipo V. Activation of NMDA receptors induces protein kinase A-mediated phosphorylation and degradation of matrin 3. Blocking these effects prevents NMDA-induced neuronal death. J Neurochem. 2005; 94:808–818. [PubMed: 16000164]

Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet. 2009; 10:32–42. [PubMed: 19065135]

Hanks SK, Hunter T. Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. FASEB J. 1995; 9:576–596. [PubMed: 7768349]

Harreman MT, Kline TM, Milford HG, Harben MB, Hodel AE, Corbett AH. Regulation of nuclear import by phosphorylation adjacent to nuclear localization signals. J Biol Chem. 2004; 279:20613–20621. [PubMed: 14998990]

Heineman TC, Seidel K, Cohen JI. The varicella-zoster virus ORF66 protein induces kinase activity and is dispensable for viral replication. J Virol. 1996; 70:7312–7317. [PubMed: 8794389]

Hobbs WE 2nd, DeLuca NA. Perturbation of cell cycle progression and cellular gene expression as a function of herpes simplex virus ICP0. J Virol. 1999; 73:8245–8255. [PubMed: 10482575]

Jans DA, Hubner S. Regulation of protein transport to the nucleus: central role of phosphorylation. Physiol Rev. 1996; 76:651–685. [PubMed: 8757785]

Kato A, Arii J, Shiratori I, Akashi H, Arase H, Kawaguchi Y. Herpes simplex virus 1 protein kinase Us3 phosphorylates viral envelope glycoprotein B and regulates its expression on the cell surface. J Virol. 2009; 83:250–261. [PubMed: 18945776]

Kato A, Tanaka M, Yamamoto M, Asai R, Sata T, Nishiyama Y, Kawaguchi Y. Identification of a physiological phosphorylation site of the herpes simplex virus 1-encoded protein kinase Us3 which regulates its optimal catalytic activity in vitro and influences its function in infected cells. J Virol. 2008; 82:6172–6189. [PubMed: 18417577]

Kato A, Yamamoto M, Ohno T, Kodaira H, Nishiyama Y, Kawaguchi Y. Identification of proteins phosphorylated directly by the Us3 protein kinase encoded by herpes simplex virus 1. J Virol. 2005; 79:9325–9331. [PubMed: 15994828]

Kato A, Yamamoto M, Ohno T, Tanaka M, Sata T, Nishiyama Y, Kawaguchi Y. Herpes simplex virus 1-encoded protein kinase UL13 phosphorylates viral Us3 protein kinase and regulates nuclear localization of viral envelopment factors UL34 and UL31. J Virol. 2006; 80:1476–1486. [PubMed: 16415024]

Kinchington PR, Fite K, Seman A, Turse SE. Virion association of IE62, the varicella-zoster virus (VZV) major transcriptional regulatory protein, requires expression of the VZV open reading frame 66 protein kinase. J Virol. 2001; 75:9106–9113. [PubMed: 11533174]

Kinchington PR, File K, Turse SE. Nuclear accumulation of IE62, the varicella-zoster virus (VZV) major transcriptional regulatory protein, is inhibited by phosphorylation mediated by the VZV open reading frame 66 protein kinase. J Virol. 2000; 74:2265–2277. [PubMed: 10666257]

Kinchington PR, Turse SE. Regulated nuclear localization of the varicella-zoster virus major regulatory protein, IE62. J Infect Dis. 1998; 178(Suppl 1):S16–S21. [PubMed: 9852967]

Klupp BG, Granzow H, Mettenleiter TC. Effect of the pseudorabies virus US3 protein on nuclear membrane localization of the UL34 protein and virus egress from the nucleus. J Gen Virol. 2001; 82:2363–2371. [PubMed: 11562530]

Koppers-Lalic D, Verweij MC, Lipinska AD, Wang Y, Quinten E, Reits EA, Koch J, Loch S, Marcondes Rezende M, Daus F, Bienkowska-Szewczyk K, Osterrieder N, Mettenleiter TC, Heemskerk MH, Tampe R, Neefjes JJ, Chowdhury SI, Ressing ME, Rijsewijk FA, Wiertz EJ. Varicellovirus UL 49.5 proteins differentially affect the function of the transporter associated with antigen processing, TAP. PLoS Pathog. 2008; 4(5):e1000080. [PubMed: 18516302]

Ku CC, Besser J, Abendroth A, Grose C, Arvin AM. Varicella-Zoster virus pathogenesis and immunobiology: new concepts emerging from investigations with the SCIDhu mouse model. J Virol. 2005; 79:2651–2658. [PubMed: 15708984]

Leach N, Bjerke SL, Christensen DK, Bouchard JM, Mou F, Park R, Baines J, Haraguchi T, Roller RJ. Emerin is hyperphosphorylated and redistributed in herpes simplex virus type 1-infected cells in a manner dependent on both UL34 and US3. J Virol. 2007; 81:10792–10803. [PubMed: 17652388]

Liang L, Roizman B. Expression of gamma interferon-dependent genes is blocked independently by virion host shutoff RNase and by US3 protein kinase. J Virol. 2008; 82:4688–4696. [PubMed: 18321964]

Lungu O, Panagiolidis CA, Annunziato PW, Gershon AA, Silverstein SJ. Aberrant intracellular localization of Varicella-Zoster virus regulatory proteins during latency. Proc Natl Acad Sci USA. 1998; 95:7081–7085.

McGeoch DJ, Davison AJ. Alphaherpesviruses possess a gene homologous to the protein kinase gene family of eukaryotes and retroviruses. Nucleic Acids Res. 1986; 14:1765–1777. [PubMed: 3005981]

Moffat JF, Zerboni L, Sommer MH, Heineman TC, Cohen JI, Kaneshima IL, Arvin AM. The ORF47 and ORF66 putative protein kinases of varicella-zoster virus determine tropism for human T cells and skin in the SCID-hu mouse. Proc Natl Acad Sci USA. 1998; 95:11969–11974. [PubMed: 9751774]

Mou F, Forest T, Baines JD. US3 of herpes simplex virus type 1 encodes a promiscuous protein kinase that phosphorylates and alters localization of lamin A/C in infected cells. J Virol. 2007; 81:6459–6470. [PubMed: 17428859]

Mou F, Wills E, Baines JD. Phosphorylation of the U(L)31 protein of herpes simplex virus 1 by the U(S)3-encoded kinase regulates localization of the nuclear envelopment complex and egress of nucleocapsids. J Virol. 2009; 83:5181–5191. [PubMed: 19279109]

Murata T, Goshima F, Daikoku T, Takakuwa H, Nishiyama Y. Expression of herpes simplex virus type 2 US3 affects the Cdc42/Rac pathway and attenuates c-Jun N-terminal kinase activation. Genes Cells. 2000; 5:1017–1027. [PubMed: 11168588]

Murata T, Goshima F, Nishizawa Y, Daikoku T, Takakuwa H, Ohtsuka K, Yoshikawa T, Nishiyama Y. Phosphorylation of cytokeratin 17 by herpes simplex virus type 2 US3 protein kinase. Microbiol Immunol. 2002; 46:707–719. [PubMed: 12477250]

Ng TI, Keenan L, Kinchington PR, Grose C. Phosphorylation of varicella-zoster virus open reading frame (ORF) 62 regulatory product by viral ORF 47-associated protein kinase. J Virol. 1994; 68:1350–1359. [PubMed: 8107200]

Nguyen ML, Kraft RM, Blaho JA. Susceptibility of cancer cells to herpes simplex virus-dependent apoptosis. J Gen Virol. 2007; 88:1866–1875. [PubMed: 17554017]

Pflum MK, Tong JK, Lane WS, Schreiber SL. Histone deacetylase 1 phosphorylation promotes enzymatic activity and complex formation. J Biol Chem. 2001; 276:47733–47741. [PubMed: 11602581]

Poon AP, Benetti L, Roizman B. U(S)3 and U(S)3.5 protein kinases of herpes simplex virus 1 differ with respect to their functions in blocking apoptosis and in virion maturation and egress. J Virol. 2006a; 80:3752–3764. [PubMed: 16571792]

Poon AP, Gu H, Roizman B. ICP0 and the US3 protein kinase of herpes simplex virus 1 independently block histone deacetylation to enable gene expression. Proc Natl Acad Sci USA. 2006b; 103:9993–9998. [PubMed: 16785443]

Poon AP, Liang Y, Roizman B. Herpes simplex virus 1 gene expression is accelerated by inhibitors of histone deacetylases in rabbit skin cells infected with a mutant carrying a cDNA copy of the infected-cell protein no. 0. J Virol. 2003; 77:12671–12678. [PubMed: 14610189]

Poon AP, Roizman B. Mapping of key functions of the herpes simplex virus 1 U(S)3 protein kinase: the U(S)3 protein can form functional heteromultimeric structures derived from overlapping truncated polypeptides. J Virol. 2007; 81:1980–1989. [PubMed: 17151133]

Purves FC, Ogle WO, Roizman B. Processing of the herpes simplex virus regulatory protein alpha 22 mediated by the UL13 protein kinase determines the accumulation of a subset of alpha and gamma mRNAs and proteins in infected cells. Proc Natl Acad Sci USA. 1993; 90:6701–6705. [PubMed: 8393574]

Purves FC, Spector D, Roizman B. The herpes simplex virus 1 protein kinase encoded by the US3 gene mediates posttranslational modification of the phosphoprotein encoded by the UL34 gene. J Virol. 1991; 65:5757–5764. [PubMed: 1656069]

Rahaus M, Desloges N, Wolff MH. Varicella-zoster virus requires a functional P13K/Akt/GSK-3 alpha/beta signaling cascade for efficient replication. Cell Signal. 2007; 19:312–320. [PubMed: 16934436]

Reynolds AE, Ryckman BJ, Baines JD, Zhou Y, Liang L, Roller RJ. U(L)31 and U(L)34 proteins of herpes simplex virus type 1 form a complex that accumulates at the nuclear rim acid is required for envelopment of nucleocapsids. J Virol. 2001; 75:8803–8817. [PubMed: 11507225]

Reynolds AE, Wills EG, Roller RJ, Ryckman BJ, Baines JD. Ultrastructural localization of the herpes simplex virus type 1 UL31, UL34, and US3 proteins suggests specific roles in primary envelopment and egress of nucleocapsids. J Virol. 2002; 76:8939–8952. [PubMed: 12163613]

Ruyechan WT, Peng H, Yang M, Hay J. Cellular factors and IE62 activation of VZV promoters. J Med Virol. 2003; 70(Suppl 1):S90–S94. [PubMed: 12627495]

Ryckman BJ, Roller RJ. Herpes simplex virus type 1 primary envelopment: UL34 protein modification and the US3-UL34 catalytic relationship. J Virol. 2004; 78:399–412. [PubMed: 14671121]

Schaap-Nutt A, Sommer M, Che X, Zerboni L, Arvin AM. ORF66 protein kinase function is required for T-cell tropism of varicella-zoster virus in vitro. J Virol. 2000; 80:11806–11816. [PubMed: 16971426]

Schaap A, Fortin JF, Sommer M, Zerboni L, Stamatis S, Ku CC, Nolan GP, Arvin AM. T-cell tropism and the role of ORF66 protein in pathogenesis of varicella-zoster virus infection. J Virol. 2005; 79:12921–12933. [PubMed: 16188994]

Schumacher D, McKinney C, Kaufer BB, Osterrieder N. Enzymatically inactive U(S)3 protein kinase of Marek's disease virus (MDV) is capable of depolymerizing F-actin but results in accumulation of virions in perinuclear invaginations and induced virus growth. Virology. 2008; 375:37–47. [PubMed: 18304599]

Schumacher R, Tischer BK, Trapp S, Osterrieder N. The protein encoded by the US3 orthologue of Marek's disease virus is required for efficient de-envelopment of perinuclear virions and involved in actin stress fiber breakdown. J Virol. 2005; 79:3987–3997. [PubMed: 15767401]

Schwer B, Verdin E. Conserved metabolic regulatory functions of sirtuins. Cell Metab. 2008; 7:104–112. [PubMed: 18249170]

Sedlackova L, Rice SA. Herpes simplex virus type 1 immediate-early protein ICP27 is required for efficient incorporation of ICP0 and ICP4 into virions. J Virol. 2008; 82:268–277. [PubMed: 17959681]

Smith-Donald BA, Roizman B. The interaction of herpes simplex virus 1 regulatory protein ICP22 with the cdc25C phosphatase is enabled in vitro by viral protein kinases US3 and UL13. J Virol. 2008; 82:4533–4543. [PubMed: 18272572]

Smith GA, Enquist LW. Break ins and break outs: viral interactions with the cytoskeleton of Mammalian cells. Annu Rev Cell Dev Biol. 2002; 18:135–161. [PubMed: 12142276]

Soong W, Schultz JC, Patera AC, Summer MH, Cohen JJ. Infection of human T lymphocytes with varicella-zoster virus: an analysis with viral mutants and clinical isolates. J Virol. 2000; 74:1864–1870. [PubMed: 10644359]

Stevenson D, Colman KL, Davison AJ. Characterization of the putative protein kinases specified by varicella-zoster virus genes 47 and 66. J Gen Virol. 1994; 75(Pt 2):317–326. [PubMed: 8113753]

Van den Broeke C, Deruelle M, Nauwynck HJ, Coller KE, Smith GA, Van Doorsselaere J, Favoreel HW. The kinase activity of pseudorabies virus US3 is required for modulation of the actin cytoskeleton. Virology. 2009a; 385:155–160. [PubMed: 19136132]

Van den Broeke C, Radu M, Deruelle M, Nauwynck H, Hofmann C, Jaffer ZM, Chernoff J, Favoreel HW. Alphaherpesvirus US3-mediated reorganization of the actin cytoskeleton is mediated by group A p21-activated kinases. Proc Natl Acad Sci USA. 2009b; 106:8707–8712. [PubMed: 19435845]

Van Minnebruggen G, Favoreel HW, Jacobs L, Nauwynck HJ. Pseudorabies virus US3 protein kinase mediates actin stress fiber breakdown. J Virol. 2003; 77:9074–9080. [PubMed: 12885923]

Wagenaar F, Pol JM, Peeters B, Gielkens AL, de Wind N, Kimman TG. The US3-encoded protein kinase from pseudorabies virus affects egress of virions from the nucleus. J Gen Virol. 1995; 76(Pt 7):1851–1859. [PubMed: 9049392]

Walters MS, Erazo A, Kinchington PR, Silverstein S. Histone deacetylases 1 and 2 are phosphorylated at novel sites during varicella-zoster virus infection. J Virol. 2009; 83:11502–11513. [PubMed: 19740981]

Wang ZX, Wu JW. Autophosphorylation kinetics of protein kinases. Biochem J. 2002; 368:947–952. [PubMed: 12190618]

Wisner TW, Wright CC, Kato A, Kawaguchi Y, Mou F, Baines JD, Roller RJ, Johnson DC. Herpesvirus γB-induced fusion between the virion envelope and outer nuclear membrane during virus egress is regulated by the viral US3 kinase. J Virol. 2009; 83:3115–3126. [PubMed: 19158241]

Yang M, Hay J, Ruyechan WT. Varicella-zoster virus IE62 protein utilizes the human mediator complex in promoter activation. J Virol. 2008; 82:12154–12163. [PubMed: 18842726]

Zeitz MJ, Malyavantham KS, Seifert B, Berezney R. Matrin 3: chromosomal distribution and protein interactions. J Cell Biochem. 2009; 108(1):125–133. [PubMed: 19562669]

Zhang Z, Carmichael GG. The fate of dsRNA in the nucleus: a p54(nrb)-containing complex mediates the nuclear retention of promiscuously A-to-I edited RNAs. Cell. 2001; 106:465–475. [PubMed: 11525732]

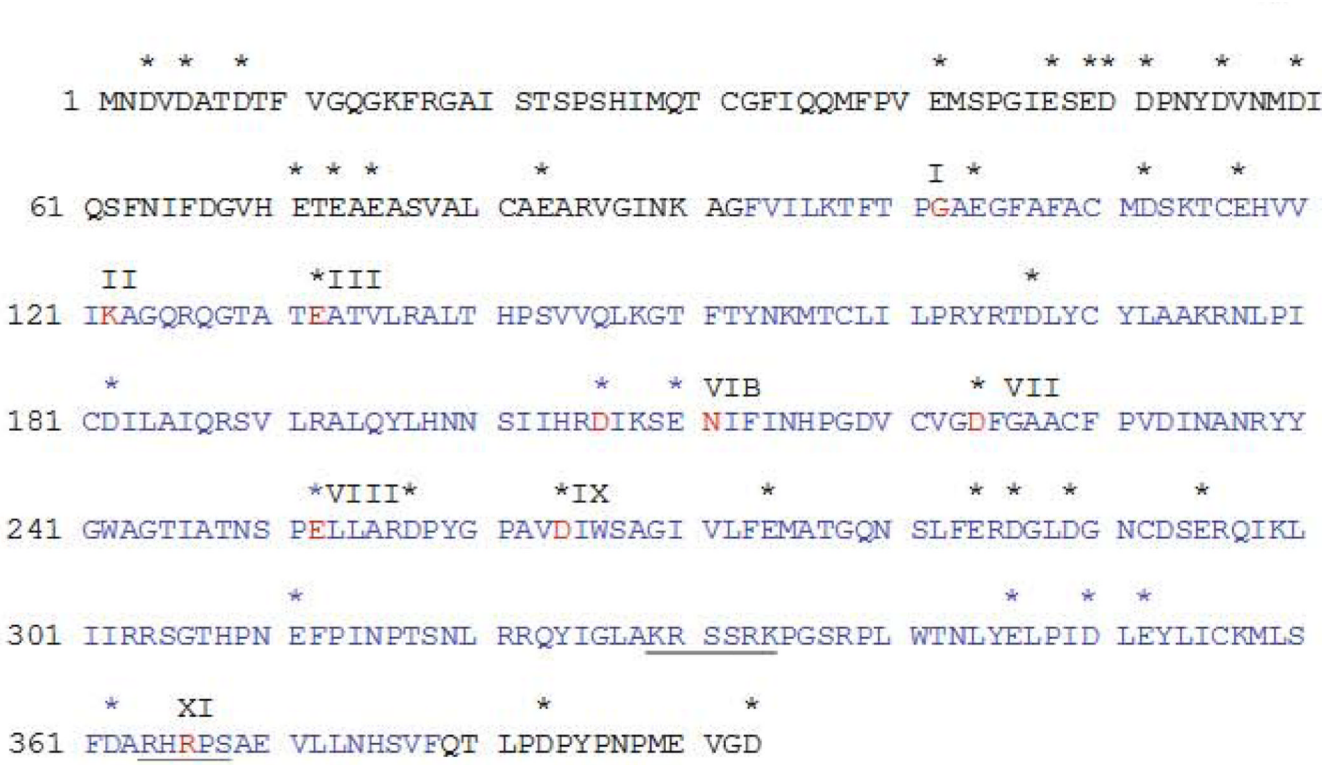

Fig. 1.

ORF66 kinase protein sequence. Catalytic domain of ORF66 is highlighted in blue. Letters highlighted in red include the nonvariant resides found amongst kinase domains indicated by the Roman numeral above the residues, which are conserved for all US3 kinases. Not all 12 kinase domains are represented. The catalytic loop is represented in italics and potential autophosphorylation target sites are underlined. Acidic residues are starred

Table 1

In vivo and in vitro proteins substrates of the ORF66/US3 kinases

Alphaherpesvirus	ORF66/US3 phosphorylated protein substrate	In vivo/ in vitro target?	Function
VZV (Eisfeld et al. 2006; Erazo et al. 2008; Kinchington et al. 2000; Kinchington and Turse 1998)	IE62 (ICP4)	+/+	IE62 cytoplasmic accumulation/IE62 tegument inclusion
VZV, HSV, PRV	Matrin 3	+/ND	?
VZV (Walters et al. 2009), HSV-1 (Poon and Roizman 2007)	HDAC 1 and 2	+/ND	Block HDAC transcriptional repression
HSV-1 (Kato et al. 2009; Wisner et al. 2009)	γB	+/+	Downregulate γB surface expression, promote virion nuclear egress
HSV-1 (Kato et al. 2005; Mou et al. 2009)	UL31	+/+	Promote virion nuclear egress
HSV-1 (Kato et al. 2005; Purves et al. 1991; Ryckman and Roller 2004)	UL34	+/+	?
HSV-1 (Mou et al. 2007)	Lamin A/C	+/+	Disrupt nuclear lamina, promote virion nuclear egress
HSV-1 (Leach et al. 2007)	Emerin	+/ND	Disrupt nuclear lamina, promote virion nuclear egress
HSV1,2 (Daikoku et al. 1994; Kato et al. 2005)	US9	+?/+	?
HSV-1 (Kato et al. 2005; Purves et al. 1993; Smith-Donald and Roizman 2008)	ICP22	+?/+	?
HSV-2 (Daikoku et al. 1995)	UL12	ND/+	?
HSV-2 (Murata et al. 2002)	Cytokeratin 17	+?/+	Cell morphological changes
HSV-1 (Cartier et al. 2003b; Kato et al. 2005)	Bad	+/+	Block apoptosis
HSV-1 (Cartier et al. 2003a; Kato et al. 2005)	Bid	ND/+/−	Block apoptosis, mediate protection from granzyme B cleavage of Bid
HSV-1 (Liang and Roizman 2008)	IFNRa	+/ND	Inhibit activation of IFN-γ genes
HSV-1 (Benetti and Roizman 2004)	PKA	+/ND	PKA activation
PRV (Van den Broeke et al. 2009b)	PAK1 and PAK2	+/+	Actin projection formation and stress fiber dissasembly
HSV-1 (Benetti and Roizman 2007)	Procaspase 3	ND/+	Block activation of procaspase 3 and apoptosis
HSV-1 (Smith-Donald and Roizman 2008)	cdc25C Phosphatase	ND/+	Enhance interaction with ICP22, optimize viral gene expression

Specific US3 kinase and its protein target are noted. Evidence of US3 induced phosphorylation in vivo or in vitro is denoted by a plus (+) sign or not determined (ND). Functions associated with phosphorylation of each protein are also listed