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Non-axial View of the Varicella-Zoster Virus Portal Protein Reveals Conserved Crown, Wing and Clip Architecture

Robert J. Visalli[*,‡] and **Alexander J. Howard**[§]

[‡]Department of Biomedical Sciences, Mercer University School of Medicine, Savannah, Georgia 31405, USA

[§]Department of Biology, Indiana University Purdue University Fort Wayne, Fort Wayne, Indiana 46805, USA

Abstract

Background—The *Herpesviridae* encode a family of protein homologs that function as the "port of entry" for insertion of the viral DNA into preformed capsids during encapsidation.

Methods—Transmission electron microscopy of recombinant Varicella-zoster virus pORF54.

Results—Results suggest that pORF54 forms higher order structures with itself. Enriched fractions analyzed by TEM revealed non-axial oriented portals with defined central channels and distinguishable crown and tail regions.

Conclusion—These morphological features are consistent with those previously reported for other herpesvirus and bacteriophage portal proteins.

Herpesviruses are enveloped, double stranded DNA viruses that infect both vertebrate and invertebrate animals. Currently eight different herpesviruses are known to cause disease in humans. All herpesviruses establish latent infections for the lifetime of the host. Human herpesviruses (HHVs) have a distinct tropism for nervous and lymphoid cells, where the body's immune surveillance is limited and the virus can remain undetected. This typically results in cycles of active viral replication (reactivation) and latent periods of infection.

Vaccination has proven valuable for VZV and will likely prove useful for the other HHVs in the future. Despite intense efforts by the biomedical research community, there is no effective cure for any established herpesvirus infection. HHVs, including our model organism, Varicella-zoster virus, can maintain a latent presence in human populations. As a consequence of latency, therapies which inhibit viral replication cannot effectively eliminate infection. Until a means of resolving latent infection is found, replication inhibitors (i.e. acyclovir) will remain the treatment of choice for suppressing and controlling symptoms of recurrent disease.

Most of the available inhibitors share the same mechanism of action, targeting viral DNA polymerase and interfering with DNA synthesis. These drugs include acyclovir, ganciclovir,

[*]Corresponding Author Robert J. Visalli, PhD, Associate Professor of Microbiology, Mercer University School of Medicine-Savannah Campus, 4700 Waters Avenue, Savannah, GA 31404, Visalli_RJ@mercer.edu, Office Phone: (912) 350-1752, Fax: (912) 350-1763.

penciclovir, brivudin, cidofovir, and foscarnet. They can be categorized into two chemical classes: nucleoside analogues and pyrophosphate analogues [1]. Following phosphorylation of their respective prodrug form, these compounds act as a substrate for viral DNA polymerase and once incorporated into the nascent DNA chain, block strand elongation. Some of these drugs have severe negative side effects, limited viral specificity, and poor bioavailability and/or toxicity profiles. Additionally, since all of these drugs share the same target, development of resistance is possible. Resistant strains of HHVs have been identified for all currently approved drugs [1].

Although therapeutic options are available for certain herpesviruses, shortcomings due to specificity, bioavailability, host toxicity and drug resistance warrant continued research aimed at identifying and developing novel therapies. Proteins that play a role in herpesviral DNA encapsidation have become promising novel chemotherapeutic targets. Two series of related non-nucleoside compounds, N-α-methylbenzyl-N'-aryl thiourea analogs, that inhibit either HSV-1 [1, 2] or VZV [1, 3] DNA encapsidation, have been described. In the presence of thiourea inhibitors, only B-capsids were observed in the nuclei of HSV or VZV infected cells. Electron microscopy revealed a lack of DNA-filled capsids in the nucleus for HSV-1 or VZV infected cells treated with their respective thiourea inhibitor [2, 3]. HSV and VZV mutant viruses resistant to thiourea compounds were found to contain mutations in their putative portal proteins, pUL6 and pORF54 respectively [2, 3]. In a separate study, the HSV-1 portal protein homolog, pUL6, was shown to be the likely target of the HSV-1 specific thiourea compounds [4]. Previously, pUL6 was shown to localize to a single vertex of the viral capsid and is the likely site of entry for viral genomic DNA during the encapsidation process [5, 6]. Additionally, HSV-1 UL6 deletion mutants are defective in both DNA cleavage and packaging, which results in large numbers of B-capsids in the nuclei of mutant-infected cells. The effect of inhibiting pUL6 or pORF54 function via the thiourea compounds is consistent with the genetic evidence provided by studies with HSV-1 deletion mutants [7, 8]. Thus, a thorough understanding of the interactions between herpesvirus portal proteins and thiourea compounds is of significant interest in the context of developing novel drug treatments for any of the herpesviruses.

Herpesviruses and dsDNA bacteriophages both utilize a common process to package their viral genomes into empty procapsids during replication. The specific chain of events following assembly of the procapsid and preceding egress of the viral particle from the nucleus (for herpesviruses [9]) or cells (in bacteriophage [10]) is known as DNA encapsidation. The packaging of viral DNA into procapsids is a critical process involving the coordinated interactions of several viral proteins. These include the portal protein, which is located at a single 5-fold vertex of the procapsid, and a complex of several other proteins known as the terminases. The terminase complex binds and cleaves viral DNA into single genome lengths while interacting directly with the portal protein to translocate viral DNA into the procapsid in an ATP dependent manner.

The eight portal homologs in human herpesviruses range in molecular mass from 68.0 kDa in HHV-8 to 86.8 kDa in VZV (Table 1A). With the exception of VZV, viruses of the same subfamily tend to have portal proteins of similar mass, most notably the gamma herpesviruses EBV (68.4 kDa) and HHV-8 (68.0 kDa). All of the herpesviruses contain a

conserved central core that is in part responsible for the amino acid homology observed between viruses of all subfamilies (Table 1B, Figs. S1 and S2). For example, VZV pORF54 amino acid identity ranges from a low of 42% with HCMV to a high of 64% with HSV-1.

It is reasonable to speculate that pORF54 performs a functional role similar to that of pUL6 and other HHV portal proteins as pORF54 shows 44% amino acid identity with its HSV-1 homolog (Table 1B) [1]. These data, in addition to the similar results observed via electron microscopy for inhibitor treated, infected cells [2, 3] are predictive of conserved functions for HSV-1 pUL6, VZV pORF54, and the other HHV portals. The location and arrangement of multimeric pUL6 has been resolved within a unique 5-fold vertex of HSV-1 B capsids. More extensive studies of bacteriophage portals have produced X-ray crystal structures of dodecameric portal proteins for bacteriophage SPP1 [11, 12]. Based on previous studies for the HSV-1 and HCMV portal proteins [13, 14] it is reasonable to assume pORF54 can self-assemble into larger, multimeric structures. Assuming that the VZV portal behaves similarly to other herpesvirus portals, a dodecameric structure would yield a complex of greater than 1.04 MDa – the largest of the HHV portal proteins (Table 1A).

Previously we performed TEM on purified pORF54 expressed in a recombinant baculovirus system. It was the first report of the Varicella-zoster virus portal protein [15]. However, we were not able to obtain any non-axial orientations of the portal complex. In this study, samples of the previously isolated pORF54 were diluted in 1.0 M arginine buffer at room temperature and gently sonicated with a probe sonicator. TEM revealed a more even distribution of portals, i.e. less aggregation and elimination of portal “balls”, and showed structural features of the VZV portal not observed previously.

Transmission electron microscopy of purified, diluted, and sonicated VZV pORF54 stained with 1% PTA on glow discharged grids is shown in Fig. 1.

Individual portal complexes with non-axial perspectives were observed. Three examples of typical portal proteins, with sizes ~25 nm in diameter are shown (Fig. 1A-C). The central channel is visible in two of the three portals (left and center panels), while the last presents a more lateral view (right). The crown and stem faces are indicated. In Fig. 1D, a very distinct central channel, presumably the entry point for viral DNA, was observed. The VZV portal is predicted to be larger than any other portals studied to date. This may explain why certain structural features such as the crown/wing and stem are readily visible in the three examples.

In other TEM analysis (Fig. 2), samples were stained with 1% PTA but not glow discharged. Particles were observed with a mushroom like appearance consistent with the predicted multimeric structure of phage portal proteins for which crystallographic data exists (Fig. 2A). Fig. 2B shows contrast enhanced images of non-glow discharged portals. The central channel can be observed in all three cases. The similarity of these images to those observed for the HCMV portal by Holzenburg et al. [13] is striking. These TEM studies represent the most revealing examples to date of intact VZV portal proteins.

A number of unique non-nucleoside alphaherpesvirus inhibitors have been identified. Novel small molecules that target VZV capsid formation [16] and the HSV helicase-primase [17-19] are under study. Further investigation of herpesvirus portals and their associated

terminase proteins will likely yield new targets for antiviral drug development. The identification of encapsidation specific antiviral inhibitors for HSV-1, HCMV, and VZV suggests that the encapsidation process is a valid antiviral target for herpesviral chemotherapy [1-3, 20, 21]. It will be interesting to examine portal formation in the presence of inhibitors in order to identify the precise mechanism of action of the thiourea inhibitor series. Future studies will focus on the effects of inhibitor treatment on the functional and structural characteristics of VZV portal protein.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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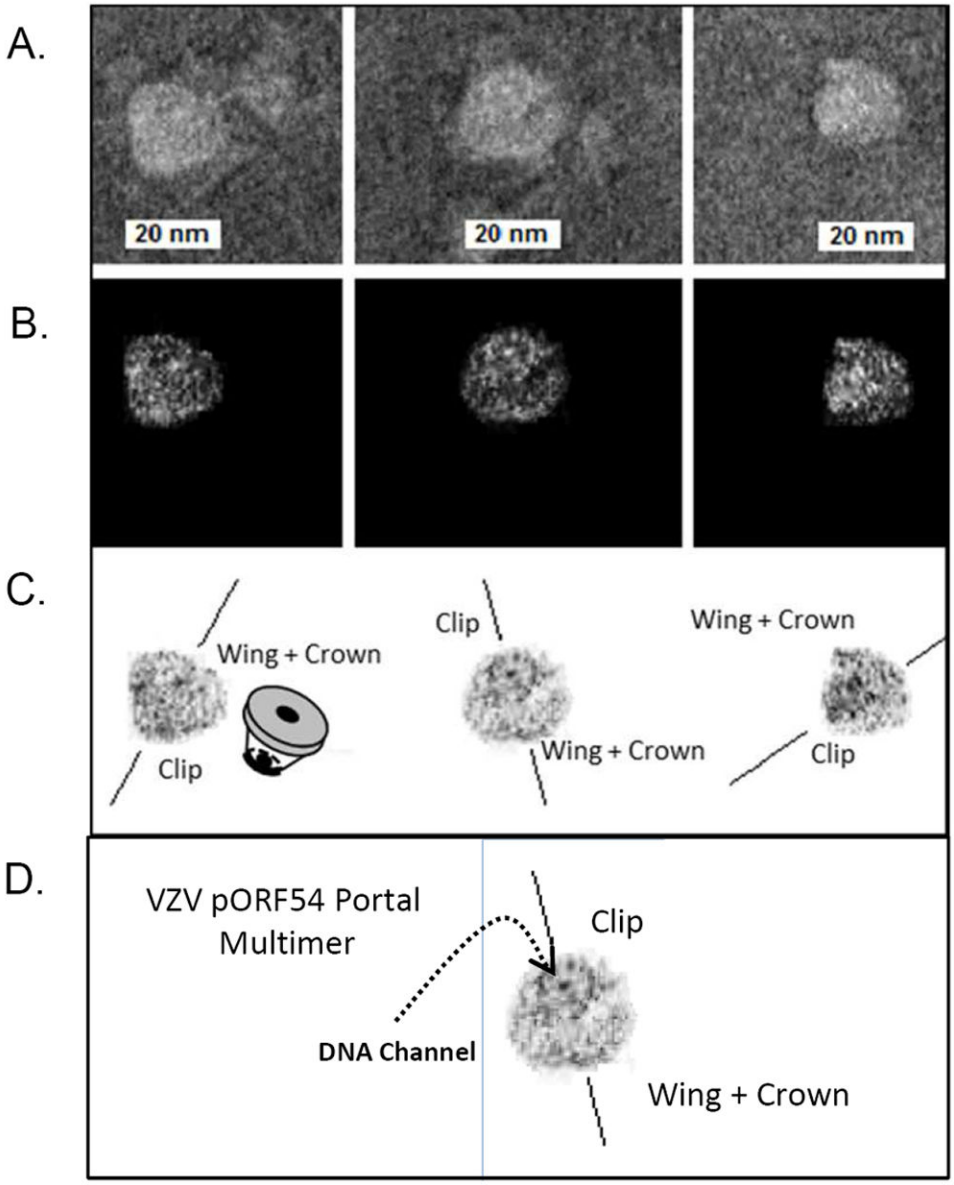

Fig. 1.

TEM of purified VZV pORF54 stained with 1% PTA, glow-discharged and imaged at 100kV using a Philips CM-100 TEM. Individual portals with non-axial perspectives. (A) Untouched images. The central channel is visible in two of the three portals (middle and left) portals, while the last presents a more lateral view (right). (B) Contrast enhanced view, with back-ground noise removed. (C) Contrast enhanced negative images with background noise removed. The crown and stem faces are indicated. (D) DNA channel is visible in the center of the portal multimer.

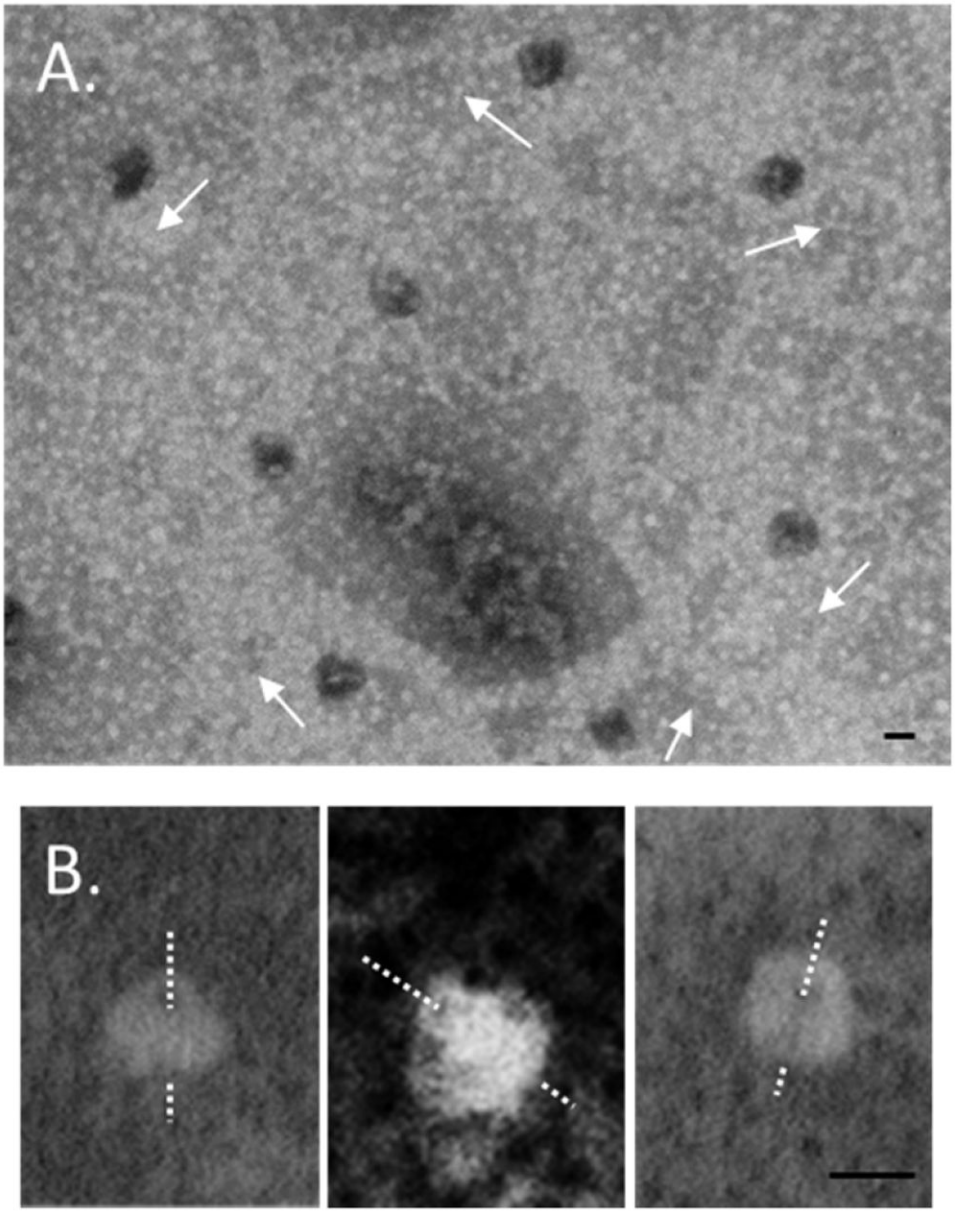

Fig. 2.

TEM of VZV portal images stained with 1% PTA without glow-discharge. (A) Portals were observed in multiple non-axial orientations. The crown/wing versus the clip can be distinguished in most cases. Arrows indicate similar orientation of the portals. (B) Contrast-enhanced, reverse-images of non-glow-discharged portal proteins. The dashed lines indicate the approximate channel path. Bars = 25 nm.

Table 1

Portal protein homologs of human herpesviruses.

A. Characteristics						
Virus	**Subfamily**	**Portal**	**Monomer (kDa)**	**Length (AA)**	**Portal Complex Mass (Mda)**	**Accession**
HSV-1	α	pUL6	74.1	676	0.889	ADM22788
HSV-2	α	pUL6	74.9	678	0.899	AEV91344
VZV	α	pORF54	86.8	769	1.042	ABJ98891
HCMV	β	pUL104	78.5	697	0.942	F5HBR4
HHV-6a	β	pU76	77.2	662	0.926	P52453
HHV-7	β	pU76	74.7	640	0.896	P52455
EBV	γ	pBBRF1	68.4	613	0.821	AFY97948
HHV-8	γ	pORF43	68	605	0.816	AAC57125

B. Sequence homology*							
Virus	**HSV-1**	**HSV-2**	**VZV**	**HCMV**	**HHV-6a**	**HHV-7**	**EBV**
HSV-1							
HSV-2	85 / 88						
VZV	44 / 64	43 / 63					
HCMV	24 / 39	25 / 40	23 / 42				
HHV-6a	23 / 42	24 / 43	24 / 44	36 / 57			
HHV-7	22 / 42	23 / 41	22 / 43	37 / 60	59 / 77		
EBV	23 / 41	24 / 41	25 / 43	24 / 41	24 /46	27 / 47	
HHV-8	23 / 41	23 / 42	25 / 45	22 / 41	27 / 49	26 / 48	51 / 70

*Amino acid identity / similarity. The amount of shading indicates level of homology between pairs of viruses.