Published in final edited form as:

Curr Top Microbiol Immunol. 2010 ; 342: 229–241. doi:10.1007/82_2009_2.

Molecular Characterization of Varicella Zoster Virus in Latently Infected Human Ganglia: Physical State and Abundance of VZV DNA, Quantitation of Viral Transcripts and Detection of VZV-Specific Proteins

Yevgeniy Azarkh, Don Gilden, and Randall J. Cohrs

Departments of Neurology (Y.A., D.G., R.J.C.) and Microbiology (D.G.), University of Colorado Denver School of Medicine, 12700 E. 19th Avenue, Mail Stop B182, Aurora. CO 80045, USA

Randall J. Cohrs: randall.cohrs@ucdenver.edu

Abstract

Varicella zoster virus (VZV) establishes latency in neurons of human peripheral ganglia where the virus genome is most likely maintained as a circular episome bound to histones. There is considerable variability among individuals in the number of latent VZV DNA copies. The VZV DNA burden does not appear to exceed that of herpes simplex type 1 (HSV-1). Expression of VZV genes during latency is highly restricted and is regulated epigenetically. Of the VZV open reading frames (ORFs) that have been analyzed for transcription during latency using cDNA sequencing, only ORFs 21, 29, 62, 63, and 66 have been detected. VZV ORF 63 is the most frequently and abundantly transcribed VZV gene detected in human ganglia during latency, suggesting a critical role for this gene in maintaining the latent state and perhaps the early stages of virus reactivation. The inconsistent detection and low abundance of other VZV transcripts suggest that these genes play secondary roles in latency or possibly reflect a subpopulation of neurons undergoing VZV reactivation. New technologies, such as GeXP multiplex PCR, have the sensitivity to detect multiple low abundance transcripts and thus provide a means to elucidate the entire VZV transcriptome during latency.

1 Introduction

After primary infection, varicella zoster virus (VZV) establishes a life-long latent infection in the ganglia of the peripheral nervous system. Cranial nerve and dorsal root ganglia (DRG) are typical sites of VZV latency, although the incidence of latent infection in trigeminal ganglia (TG) is higher than in any single DRG (Gilden et al. 1983; Mahalingam et al. 1992). VZV DNA has also been detected in celiac and nodose ganglia of the autonomic nervous system (Gilden et al. 2001). Under conditions of decreased adaptive immunity (aging, HIV infection or organ transplantation), VZV can reactivate to cause herpes zoster, frequently complicated by postherpetic neuralgia, myelopathy, vasculopathy, and retinal necrosis (Arvin 1996; Nagel and Gilden 2007).

Efforts to identify new zoster therapeutics have included extensive research on the molecular events involved in VZV latency and reactivation. However, the understanding of VZV latency and reactivation lags behind that of herpes simplex type 1 (HSV-1; the prototypic neurotropic alphaherpesvirus) primarily due to the highly cell-associated nature

of VZV and the lack of a suitable small-animal model. This review summarizes current knowledge of VZV DNA, RNA, and proteins in human tissue obtained at autopsy.

2 Ganglionic Cell Type Infected by VZV

Initial reports on the location of VZV within human ganglia during latency were based on the detection of viral transcripts by in situ hybridization (ISH) and led to conflicting conclusions. Using ISH, Hyman et al. (1983) found VZV RNA exclusively in neurons of TG, and Gilden et al. (1987) found VZV nucleic acids in neurons of thoracic ganglia, whereas subsequent reports by Croen et al. (1988) and Meier et al. (1993) indicated the presence of VZV transcripts exclusively in non-neuronal cells. Later, in situ PCR amplification and hybridization with digoxigenin-labeled probes revealed VZV DNA predominately in the nuclei of latently infected neurons (Dueland et al. 1995; Kennedy et al. 1998). As an alternative to ISH, a technology prone to errors arising from individual interpretation (Mahalingam et al. 1999), LaGuardia et al. (1999) used PCR to analyze VZV DNA in latently infected human ganglia after separation into neuronal and non-neuronal cell fractions by differential filtration; VZV DNA was detected in 9 of 16 samples containing an estimated 5,000 neurons, but in only 2 of 16 containing about 500,000 corresponding non-neuronal cells. In a similar experiment, Levin et al. (2003) mechanically separated ganglia into neuronal and non-neuronal cells using a micromanipulator, and again, VZV DNA was detected only in neurons; 34 of 2,226 neurons and none of 20,700 satellite cells contained VZV DNA, with an average of 4.7 copies of VZV DNA per infected neuronal cell. Wang et al. (2005), who used laser capture microdissection (LCM) and PCR to analyze a total of 1,722 neurons and 14,200 non-neuronal cells isolated from TGs of ten subjects, reported VZV DNA in 6.9% neurons and 0.06% non-neuronal cells. Overall, multiple independent studies using different methods indicate that VZV resides predominantly in neurons during latency.

3 Distribution of VZV DNA in Ganglia

In the only known study focusing on assessing the distribution of VZV DNA in human ganglia, Cohrs et al. (2005) used PCR to examine DNA extracted from 2–4 sections of fixed TG sectioned at 100- μ m intervals. VZV DNA was found to be distributed evenly throughout the ganglia, but only in sections that contained neuronal nuclei.

4 Latent VZV Burden

The latent VZV DNA burden per 100,000 ganglionic cells has been variously estimated at 6–31 VZV genome copies (Mahalingam et al. 1993), 258 ± 38 copies (Pevenstein et al. 1999), and $9,046 \pm 13,225$ copies (Cohrs et al. 2000). The 35-fold discrepancy between the latter two reports is curious, since both studies were based on real-time quantitative PCR, and both detected similar amounts of HSV-1 DNA in TGs: $2,902 \pm 1,082$ copies and $3,042 \pm 3,274$ copies, respectively, per 100,000 ganglionic cells. The variability in latent VZV DNA copy number most likely reflects variations in ganglia from randomly selected individuals representing an outbred human population with decades of re-exposure to the virus. Thus, while consensus on the VZV DNA burden during latency requires analysis of ganglia from additional individuals, the numbers are unlikely to greatly exceed those found for HSV-1.

5 Configuration of Latent VZV DNA

Understanding the physical state of the VZV genome in latently infected ganglionic neurons can provide information about the molecular mechanism by which VZV latency is maintained and reactivation initiated. To examine the configuration of latent VZV DNA, Clarke et al. (1995) designed PCR primers that amplified a fragment within the unique long

segment of the VZV genome or, should the ends join, the junction between the genomic termini. PCR amplification using plasmid based standards and DNA extracted from ganglia and from VZV nucleocapsids revealed a ratio of products obtained by internal vs. terminal primers of ~1 for all ganglia ($n = 12$), but 15:1 for the virion DNA. Thus, genomic termini were joined in essentially all ganglia, while 5% of VZV DNA present in the virus contained inversion of the unique long genomic segment. This finding suggests that like HSV-1, VZV DNA assumes a circular configuration during latency, and that the mechanism by which both herpesviruses establish latency is analogous.

6 Epigenetic Regulation of Latent VZV Gene Transcription

Chromatin immunoprecipitation (ChIP) assays followed by PCR have been used to elucidate the epigenetic state of VZV genomes (Gary et al. 2006). Like HSV-1 VZV DNA is associated with histones at all stages of the viral life cycle, but the histone composition differs between productive infection and latency. For example, acetylated histone H3K9(Ac), indicative of a euchromatic (transcriptionally active) state, is associated with ORF62 and ORF63 promoters during both latent infection in human ganglia and lytic infection of human melanoma (MeWo) cells ORF62 and ORF63 are regulatory proteins of the immediate-early kinetic class that are expressed during latency. In contrast the promoters regulating transcription of VZV ORF 14 (glycoprotein C) and ORF 36 (thymidine kinase), which are not expressed in latency, are not associated with H3K9(Ac) in latent infection. Thus, chromatin remodeling appears to contribute to the restricted pattern of latent VZV gene transcription.

7 The VZV Transcriptome

Analysis of the VZV genome predicts 71 open reading frames (ORFs) encoding proteins of more than 100 amino acids (Davison and Scott 1986). Of these ORFs, 65 are present in a single copy, while the remaining 6 map within the repeated regions of the unique short segment of the virus genome and thus form three diploid ORFs. Subsequent analyses have identified ORFs 0, 9A, and 33.5 (Kemble et al. 2000; Ross et al. 1997; Chaudhuri et al. 2008), thus revealing a total of 74 ORFs, of which 71 are unique.

Determination of VZV gene expression represents a major step in understanding virus pathogenicity, and array technology has provided a broad picture of the viral transcriptome in productively infected cells. Using macroarrays with cloned PCR fragments to target both the 5' and 3' ends of all ORFs, Cohrs et al. (2003b) analyzed RNA extracted from VZV (Ellen strain)-infected BSC-1 (African green monkey kidney) cells and detected transcripts mapping to all unique VZV ORFs originally identified by Davison and Scott (1986) except ORF14 (unstable when cloned). The relative expression levels of all VZV ORFs increased uniformly from days 1 to 3 postinfection with ORFs 9/9A, 33/33.5, 49, 63/70, and 64/69 being the most abundant. Kennedy et al. (2005), who used long-oligonucleotide microarrays to analyze VZV (Dumas strain) transcription in MeWo and glial cells 72 h postinfection, found that the VZV gene transcription pattern in MeWo cells was similar to that in BSC-1 cells, but markedly different from that in glial cells, where only 20 ORFs were detected. Moreover, some ORFs that were not detected by microarray were amplified by reverse transcriptase-PCR (RT-PCR), suggesting that they are expressed in low abundance in glial cells.

Whereas macroarrays can clearly detect VZV transcripts in productively infected cells in culture (Cohrs et al. 2003b), their usefulness in assessing the extent of VZV transcription in latently infected human ganglia remains problematic due to insufficient sensitivity and the low abundance of latent VZV transcripts. Preliminary experiments showed that macroarrays

failed to detect VZV ORF 63 transcripts in mRNA extracted from latently infected TG in which nested-set PCR had revealed the presence of ORF 63 mRNA (Cohrs, personal communication). Subsequent use of microarrays in which six picoliters of PCR-generated target DNA were applied onto glass slides and hybridization was performed in 20 μ l (compared to 20 ml for macroarrays), increased the sensitivity of detection (abundance of ~10,000 copies), but ORF 63 transcripts known to be present in human ganglionic mRNA were still undetectable (Nagel, personal communication). Efforts to identify the full extent of VZV transcription during latency continue by studying the expression of individual VZV genes and by developing a more sensitive alternative to microarrays.

8 Detection and Quantitation of Individual VZV Transcripts in Latently Infected Ganglia

VZV gene expression is highly restricted during latency. Of the 71 known VZV ORFs, only ORFs 4, 10, 18, 21, 28, 29, 40, 51, 62, 63 and 66 transcripts have been examined in latently infected human ganglia by ISH, PCR amplification of cDNA libraries or by RT-PCR (Table 1). Using ISH, Kennedy et al. (2000) detected transcripts mapping to ORFs 4, 18, 21, 29, 40, 62, and 63, but not to ORFs 28 or 61 in human TG. Transcripts mapping to ORFs 21, 29, 62, 63, and 66, but not ORFs 4, 10, 40, 51, or 61 were detected in latently infected TG by PCR amplification of cDNA libraries (Cohrs et al. 1994,1996) and by RT-PCR (Cohrs et al. 2003b). To confirm that PCR products reflected amplification of viral cDNA, the 3'-polyadenylated termini of the transcripts were sequenced (Cohrs et al.1996, 2003b). Based on sequence data, ORFs 21, 29, 62, 63, and 66 are transcribed in latently infected human ganglia (Table 1). Quantitative analysis demonstrated that ORF63 is the most abundant and frequently detected VZV transcript. VZV ORF63 transcripts in individual ganglia vary over 2,000-fold from 1 to 2,785 copies per 10,000 copies of GAPdH transcript (Cohrs et al. 2000), and ORF63 transcripts were detected in 17 of 28 ganglia with the highest copy number > 29,000 copies per 1 μ g of input mRNA (Cohrs and Gilden 2003). The repeated detection and high abundance of VZV ORF63 transcripts during latency point to a critical role for this transcript in the maintenance of latency or in the early stages of virus reactivation.

9 Development of a Novel Assay to Study VZV Gene Expression During Latency

As mentioned above, current array technologies lack the sensitivity required to VZV transcripts in latently infected ganglia. As an alternative, Nagel et al (2009) adapted multiplex PCR (GeXPS; Beckman Coulter) to detect the 68 VZV ORFs originally identified by Davison and Scott (1986). GeXPS technology is based on simultaneous reverse transcription of a single mRNA pool with VZV gene-specific primers followed by PCR amplification of the multiple cDNA targets using primers specific for each cDNA. The key to GeXPS analysis is the use of chimeric PCR primers that contain both gene-specific and universal DNA sequences. Amplification of the cDNA sample with optimized concentrations of these primers results in an unbiased pool of products of predetermined length, each containing a fluorescent marker on the 5' end. Capillary electrophoresis is then used to separate the PCR products based on size and to determine the abundance of each product based on a fluorescent signal. Since the abundance of a PCR product depends on primer amplification efficiency, it is not quantitative; however, GeXPS technology allows comparison of transcript abundance in a sample-to-sample fashion, and permits screening of multiple samples in a high-throughput format. Applying GeXPS technology, Nagel et al. (2009) detected transcripts from all VZV genes in only five PCR reactions. Parallel analysis of RNA serial dilutions by GeXPS and real-time PCR showed that GeXPS multiplex

analysis was sufficiently sensitive to detect as little as 20 copies of ORF 21, 29, 62, 63, and 66 transcripts. Thus, GeXPS technology can be used to determine the extent of VZV transcription in latently infected human ganglia.

10 MicroRNA Expression in Latently Infected Ganglia

MicroRNAs (miRNA) are small noncoding RNA molecules that alter transcript stability and translation when bound to their target mRNA. Deep sequencing of RNA extracted from human TG positive for VZV and HSV-1 DNA revealed several miRNAs mapping to the HSV-1 genome, but no VZV specific miRNAs (Umbach et al. 2009). Since the genetic locus containing the HSV-1 miRNA maps to the latency-associated transcript region of HSV-1, and the homologous region is deleted in VZV, the lack of VZV miRNA may reflect a basic mechanistic difference inherent in the way these neurotropic alphaherpesvirus maintain latency.

11 VZV Protein in Latently Infected Ganglia

To date, the only technique used to detect VZV proteins in latently infected ganglia has been immunohistochemistry (IHC). Proteins encoded by ORF 4, 21, 29, 62, 63, and 66 have been detected in the cytoplasm of neurons using various antibody sources (Table 1). The cytoplasmic localization of these proteins during latency, many of which are predominately nuclear during productive infection, may indicate a possible mechanism by which latency is maintained. However, the finding of proteins in ganglia without repeated detection of the respective transcript also suggests problems inherent to IHC. Just as the use of multiple independent techniques has aided the characterization of VZV transcripts in latently infected human ganglia, so too must the characterization of latently expressed VZV proteins await confirmation by independent techniques. Preliminary results suggest that Western blot analysis is not adequate to detect ORF 63 protein in ganglia containing abundant levels of ORF 63 transcripts (Cohrs, personal communication), pointing to the need for new, more sensitive technologies to detect VZV proteins during latency.

12 Future Directions

Analyses of multiple human ganglia obtained at autopsy indicate that latent VZV is predominantly, if not exclusively, located in ganglionic neurons. The VZV burden is variable, but not likely to exceed that found for HSV-1. VZV gene expression during latency is restricted, but not silenced. The studies reviewed here form a basis of our understanding of VZV latency and have helped to identify areas for future investigation:

1. VZV has been shown to establish latency in neurons (Hyman et al. 1983; Gilden et al. 1987; Cohrs et al. 2005), but is there a particular subpopulation of neurons that is selectively vulnerable to VZV infection?
2. While VZV is a remarkably stable virus, at least four distinct clades have been identified (Peters et al. 2006). Is there a correlation between specific VZV clades and the latent virus burden? Is there a demographic component to VZV latency?
3. Some latently transcribed VZV genes are regulated, in part, at the epigenetic level (Gary et al. 2006). Is this a universal feature of latent VZV gene regulation? What is the full repertoire of epigenetic markers associated with the latent virus genome? How is epigenetic regulation modified during reactivation?
4. Extensive investigation indicates that ORF 63 transcription is a hallmark of VZV latency (Cohrs and Gilden 2007); however, no complete search of the latent VZV transcriptome has been performed with the required sensitivity. Future studies

await new technologies to investigate the full spectrum of VZV transcription during latency.

5. The nuclear redistribution of VZV ORF 63 protein has been detected during VZV reactivation (Lungu et al. 1998). What is the molecular basis for cytoplasmic and nuclear localization of IE63? What is the function of cytoplasmic and nuclear IE63? Does posttranslational processing of IE63 play a role in virus reactivation?
6. No VZV-specific miRNAs are detected during latency (Umbach et al. 2009); however, herpesviruses are able to modify expression of host cell miRNAs (Wang et al. 2008). Does VZV latency similarly modify the pool of miRNA in neurons?
7. VZV transcripts other than ORF 63 have been occasionally detected at low abundance during latency (Cohrs and Gilden 2007). What is the mechanism by which these transcripts are regulated? Are these transcripts required to maintain latency? Do these transcripts reflect limited virus reactivation?
8. Finally, what is the mechanism for VZV reactivation? The answer to this question will depend on the successful development of a valid experimental model of VZV latency, the most promising of which is simian varicella virus infection of monkeys (Mahalingam et al. 2007).

Answering these questions will greatly enhance our understanding of VZV molecular biology, and will require the development of exquisitely sensitive technologies to analyze the low abundance of this fascinating virus.

Acknowledgments

This work was supported in part by Public Health Service grants NS032623 and AG032958 from the National Institutes of Health. The authors thank Dr. Robert Cordery-Cotter and Marina Hoffman for editorial review and Cathy Allen for preparing the manuscript.

Abbreviations

ChIP	Chromatin immunoprecipitation
DRG	Dorsal root ganglia
HSV	Herpes simplex virus
IHC	Immunohistochemistry
LCM	Laser capture microdissection
miRNA	MicroRNA
ORF	Open reading frame
qRT-PCR	Quantitative reverse transcriptase-PCR
RT-PCR	Reverse transcriptase-PCR
TG	Trigeminal ganglia
VZV	Varicella zoster virus

References

- Arvin A. Varicella-zoster virus. *Clin Microbiol Rev* 1996;9:161–381.
- Chaudhuri V, Sommer M, Rajamani J, et al. Functions of varicella-zoster virus ORF23 capsid protein in viral replication and the pathogenesis of skin infection. *J Virol* 2008;82:10231–10246. [PubMed: 18684828]

- Clarke P, Beer T, Cohrs R, et al. Configuration of latent varicella-zoster virus DNA. *J Virol* 1995;69:8151–8154. [PubMed: 7494340]
- Cohrs RJ, Gilden DH. Varicella zoster virus transcription in latently infected ganglia. *Anticancer Res* 2003;23:2063–2070. [PubMed: 12894579]
- 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, Srock K, Barbour MB, et al. Varicella-zoster virus (VZV) transcription during latency in human ganglia: construction of a cDNA library from latently infected human trigeminal ganglia and detection of a vzv transcript. *J Virol* 1994;68:7900–7908. [PubMed: 7966580]
- Cohrs RJ, Barbour M, Gilden DH. Varicella-zoster virus (VZV) transcription during latency in human ganglia: detection of transcripts mapping to genes 21, 29, 62, and 63 in cDNA library enriched for VZV RNA. *J Virol* 1996;70:2789–2796. [PubMed: 8627753]
- Cohrs RJ, Randall J, Smith J, et al. Analysis of individual human trigeminal ganglia for latent herpes simplex virus type 1 and varicella-zoster virus nucleic acids using real-time PCR. *J Virol* 2000;74:11464–11471. [PubMed: 11090142]
- Cohrs RJ, Gilden DH, Kinchington PR, et al. Varicella-zoster virus gene 66 transcription and translation in latently infected human ganglia. *J Virol* 2003a;77:6660–6665. [PubMed: 12767985]
- Cohrs RJ, Hurley MP, Gilden DH. Array analysis of viral gene transcription during lytic infection of cells in tissue culture with varicella-zoster virus. *J Virol* 2003b;77:11718–11732. [PubMed: 14557657]
- Cohrs RJ, LaGuardia JJ, Gilden DH. Distribution of latent herpes simplex virus type-1 and varicella zoster virus DNA in human trigeminal ganglia. *Virus Genes* 2005;31:223–227. [PubMed: 16025248]
- Croen KD, Ostrove JM, Dragovic LJ, et al. Patterns of gene expression and sites of latency in human nerve ganglia are different for varicella-zoster and herpes simplex viruses. *Proc Natl Acad Sci USA* 1988;85:9773–9777. [PubMed: 2849116]
- Davison AJ, Scott JE. The Complete DNA Sequence of varicella-zoster virus. *J Gen Virol* 1986;67:1759–1816. [PubMed: 3018124]
- Dueland AN, Ranneberg-Nilsen T, Degré M. Detection of latent varicella zoster virus DNA and human gene sequences in human trigeminal ganglia by in situ amplification combined with in situ hybridization. *Arch Virol* 1995;140:2055–2066. [PubMed: 7503701]
- Gary L, Gilden DH, Cohrs RJ. Epigenetic regulation of varicella-zoster virus open reading frames 62 and 63 in latently infected human trigeminal ganglia. *J Virol* 2006;80:4921–4926. [PubMed: 16641283]
- Gilden DH, Vafai A, Shtram Y, et al. Varicella-zoster virus DNA in human sensory ganglia. *Nature* 1987;306:478–480. [PubMed: 6316159]
- Gilden DH, Rozenman Y, Murray R, et al. Detection of varicella-zoster virus nucleic acid in neurons of normal human thoracic ganglia. *Ann Neurol* 1987;22:377–380. [PubMed: 2823688]
- Gilden DH, Gesser R, Smith J, et al. Presence of VZV and HSV-1 DNA in human nodose and celiac ganglia. *Virus Genes* 2001;23:145–147. [PubMed: 11724266]
- Gilden E, Kennedy PGE. Translation of varicella-zoster virus genes during human ganglionic latency. *Virus Genes* 2004;29:317–319. [PubMed: 15550771]
- Hüfner K, Derfuss T, Herberger S, et al. Latency of α -herpes viruses is accompanied by a chronic inflammation in human trigeminal ganglia but not in dorsal root ganglia. *J Neuropathol Exp Neurol* 2006;65:1022–1030. [PubMed: 17021407]
- Hyman RW, Ecker JR, Tenser RB. Varicella-zoster virus RNA in human trigeminal ganglia. *Lancet* 1983;2:814–816.
- Kemble GW, Annuziato P, Lungu O, et al. Open reading frame S/L of varicella-zoster virus encodes a cytoplasmic protein expressed in infected cells. *J Virol* 2000;74:11311–11321. [PubMed: 11070031]
- Kennedy PG, Grinfeld E, Gow JW. Latent varicella-zoster virus is located predominantly in neurons in human trigeminal ganglia. *Proc Natl Acad Sci USA* 1998;95:4658–4662. [PubMed: 9539794]
- Kennedy PGE, Grinfeld E, Bell JE. Varicella-zoster virus gene expression in latently infected and explanted human ganglia. *J Virol* 2000;74:11893–11898. [PubMed: 11090189]

- Kennedy PGE, Grinfeld E, Craigon M, et al. Transcriptomal analysis of varicella-zoster virus infection using long oligonucleotide-based microarrays. *J Gen Virol* 2005;86:2673–2684. [PubMed: 16186220]
- LaGuardia JJ, Cohrs RC, Gilden DH. Prevalence of varicella-zoster virus DNA in dissociated human trigeminal ganglion neurons and nonneuronal cells. *J Virol* 1999;73:8571–8577. [PubMed: 10482610]
- Levin MJ, Cai G-Y, Manchak MD, et al. Varicella-zoster virus DNA in cells isolated from human trigeminal ganglia. *J Virol* 2003;77:6979–6987. [PubMed: 12768016]
- Lungu O, Panagiotidis C, Annunziato PW, et al. Aberrant intracellular localization of varicella-zoster virus regulatory proteins during latency. *Proc Natl Acad Sci USA* 1998;95:7080–7085. [PubMed: 9618542]
- Mahalingam R, Wellish MC, Dueland AN, et al. Localization of herpes simplex virus and varicella zoster virus DNA in human ganglia. *Ann Neurol* 1992;31:444–448. [PubMed: 1316733]
- Mahalingam R, Wellish M, Lederer D, et al. Quantitation of latent varicella-zoster virus DNA in human trigeminal ganglia by polymerase chain reaction. *J Virol* 1993;67:2381–2384. [PubMed: 8383249]
- Mahalingam R, Wellish M, Cohrs R, et al. Expression of protein encoded by varicella-zoster virus open reading frame 63 in latently infected human ganglionic neurons. *Proc Natl Acad Sci USA* 1996;93:2122–2124. [PubMed: 8700895]
- Mahalingam R, Lasher R, Wellish M, et al. Localization of varicella-zoster virus gene 21 protein in virus-infected cells in culture. *J Virol* 1998;72:6832–6837. [PubMed: 9658132]
- Mahalingam R, Kennedy PGE, Gilden DH. The problems of latent varicella zoster virus in human ganglia: precise cell location and viral content. *J Neurovirol* 1999;5:445–448. [PubMed: 10568880]
- Mahalingam R, Traina-Dorge V, Wellish M, et al. Simian varicella virus reactivation in cynomolgous monkeys. *Virology* 2007;368:50–59. [PubMed: 17651776]
- Meier JL, Holman RP, Croen KD, et al. Varicella-zoster virus transcription in human trigeminal ganglia. *Virology* 1993;193:193–200. [PubMed: 7679857]
- Nagel MA, Gilden DH. The protean neurologic manifestations of varicella-zoster virus infection. *Cleve Clin J Med* 2007;74:489–504. [PubMed: 17682626]
- Nagel MA, Gilden D, Shade T, et al. Rapid and sensitive detection of 68 unique varicella zoster virus gene transcripts in five multiplex reverse transcription-polymerase chain reactions. *J Virol Methods* 2009;157:62–68. [PubMed: 19109999]
- Peters GA, Tyler SD, Grose C, et al. A full-genome phylogenetic analysis of varicella-zoster virus reveals a novel origin of replication-based genotyping scheme and evidence of recombination between major circulating clades. *J Virol* 2006;80:9850–9860. [PubMed: 16973589]
- Pevenstein SR, Williams RK, McChesney D, et al. Quantitation of latent varicella-zoster virus and herpes simplex virus genomes in human trigeminal ganglia. *J Virol* 1999;73:10514–10518. [PubMed: 10559370]
- Ross J, Williams M, Cohen JI. Disruption of the varicella-zoster virus dUTPase and the adjacent ORF9A gene results in impaired growth and reduced syncytia formation *in vitro*. *Virology* 1997;234:186–195. [PubMed: 9268149]
- Theil D, Derfuss T, Paripovic I, et al. Latent herpesvirus infection in human trigeminal ganglia causes chronic immune response. *Am J Pathol* 2003;163:2179–2184. [PubMed: 14633592]
- Tyler SD, Peters GA, Grose C, et al. Genomic cartography of varicella-zoster virus: a complete genome-based analysis of strain variability with implications for attenuation and phenotypic differences. *Virology* 2007;359:447–458. [PubMed: 17069870]
- Umbach JL, Nagel MA, Cohrs RJ, et al. Analysis of human alphaherpesviruses microRNA expression in latently infected human trigeminal ganglia. *J Virol* 2009;83:10677–10683. [PubMed: 19656888]
- Wang K, Lau TY, Morales M, et al. Laser-capture microdissection: refining estimates of the quantity and distribution of latent herpes simplex virus 1 and varicella-zoster virus DNA in human trigeminal ganglia at the single-cell level. *J Virol* 2005;79:14079–14087. [PubMed: 16254342]
- Wang F-Z, Weber F, Croce C, et al. Human cytomegalovirus infection alters the expression of cellular microRNA species that affect its replication. *J Virol* 2008;82:9065–9074. [PubMed: 18596100]

VZV gene expression in latently infected human ganglia

Table 1

ORF	Description ^d	Findings	Transcript verified by cDNA sequencing	ORF protein detected by IHC ^b	Method	Reference
4	Transactivator, tegument protein	Transcripts found in non-neuronal cells			ISH ^c	Croen et al. (1988)
		Transcripts not found			PCR of cDNA library	Cohrs et al. (1996)
		Protein found in cytoplasm of neurons		Yes	IHC	Lungu et al. (1998)
		Transcripts found in neurons			ISH	Kennedy et al. (2000)
10	Transactivator, tegument protein	Transcripts not found			PCR of cDNA library	Cohrs et al. (1996)
18	Ribonucleotide reductase, small subunit	Transcripts found in neurons			ISH	Kennedy et al. (2000)
21	HSV-1 UL37 homolog; nucleocapsid-associated protein ^d	Transcripts found	Yes		PCR of cDNA library	Cohrs et al. (1994, 1996)
		Protein found in cytoplasm of neurons		Yes	IHC	Lungu et al. (1998), Grinfeld and Kennedy (2004)
		Transcripts found and quantified			qRT-PCR ^e	Cohrs et al. (2000)
		Transcripts found in neurons			ISH	Kennedy et al. (2000)
		Transcripts not found			qRT-PCR	Cohrs and Gilden (2007)
28	DNA polymerase	Transcripts not found			ISH	Kennedy et al. (2000)
29	Single-stranded DNA-binding protein	Transcripts found in non-neuronal cells			ISH	Croen et al. (1988), Meier et al. (1993)
		Transcripts found	Yes		PCR of cDNA library	Cohrs et al. (1996)
		Protein found in cytoplasm of neurons		Yes	IHC	Lungu et al. (1998), Grinfeld and Kennedy (2004)
		Transcripts found and quantified			qRT-PCR	Cohrs et al. (2000), Cohrs and Gilden (2007)
		Transcripts found in neurons			ISH	Kennedy et al. (2000)
40	Major nucleocapsid protein	Transcripts not found			PCR of cDNA library	Cohrs et al. (1996)
		Transcripts found			ISH	Kennedy et al. (2000)
51	Helicase, origin-binding protein	Transcripts not found			PCR of cDNA library	Cohrs et al. (1996)
61	Transactivator, transrepressor	Transcripts not found			PCR of cDNA library	Cohrs et al. (1996)
		Transcripts not found			ISH	Kennedy et al. (2000)
62	Transactivator, tegument protein	Transcripts found in non-neuronal cells			ISH	Croen et al. (1988), Meier et al. (1993)
		Transcripts found	Yes		PCR of cDNA	Cohrs et al. (1996)

ORF	Description ^a	Findings	Transcript verified by cDNA sequencing	ORF protein detected by IHC ^b	Method	Reference
		Protein found in cytoplasm of neurons		Yes	IHC	Lungu et al. (1998), Theil et al. (2003), Grinfeld and Kennedy (2004), Hüfner et al. (2006)
		Transcripts found in neurons			ISH	Kennedy et al. (2000)
		Transcripts found and quantified			qRT-PCR	Cohrs and Gilden (2007)
63	Tegument protein	Transcripts found in neurons			ISH	Gilden et al. (1987), Kennedy et al. (2000)
		Transcripts found in non-neuronal cells			ISH	Croen et al. (1988)
		Transcripts found	Yes		PCR of cDNA	Cohrs et al. (1996)
		Protein found in cytoplasm of neurons		Yes	IHC	Mahalingam et al. (1996), Lungu et al. (1998), Grinfeld and Kennedy (2004)
		Transcripts found and quantified			qRT-PCR	Cohrs et al. (2000), Cohrs and Gilden (2007)
66	Serine-threonine protein kinase	Transcripts found	Yes		ISH, RT-PCR ^f	Cohrs et al. (2003a)
		Protein found in cytoplasm of neurons		Yes	IHC	Cohrs et al. (2003a)
		Transcripts found and quantified			qRT-PCR	Cohrs and Gilden (2007)

^aTyler et al. (2007)

^bimmunohistochemistry

^cIn situ hybridization

^dMahalingam et al. (1998)

^eQuantitative reverse transcriptase-PCR

^fReverse transcriptase-PCR