Varicella-Zoster Virus Open Reading Frame 21, Which Is Expressed during Latency, Is Essential for Virus Replication but Dispensable for Establishment of Latency

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Varicella-zoster virus (VZV) open reading frame 21 (ORF21) is one of at least five VZV genes expressed in latently infected human and rodent ganglia. To determine whether ORF21 is required for latent and lytic infection, we deleted 99% of ORF21 from the viral genome. The ORF21 deletion mutant virus could be propagated only in a cell line expressing the ORF21 protein. Insertion of the herpes simplex virus type 1 (HSV-1) homolog of VZV ORF21, HSV-1 UL37, into the ORF21 deletion mutant failed to complement the mutant for growth in cell culture. Inoculation of cotton rats with the ORF21 deletion virus resulted in latent infection in numbers of animals similar to those infected after inoculation with the parental virus. The mean numbers of latent VZV genomes were similar in animals infected with parental and ORF21 deletion viruses. Transcription of ORF63, another latency-associated gene, was detected in ganglia from similar numbers of animals infected with the mutant and parental viruses. Thus, ORF21 is the first VZV gene expressed during latency that has been shown to be dispensable for the establishment of latent infection.

Varicella-zoster virus (VZV) establishes a latent infection in humans in dorsal root and cranial nerve ganglia. Transcripts for five VZV genes, open reading frame 4 (ORF4), ORF21, ORF29, ORF62, and ORF63, have been detected in latently infected human ganglia by several laboratories (6, 8, 17, 26). The ORF63 protein has also been detected during latency (17, 21, 23, and other proteins, including the ORF21, ORF29, and ORF62 proteins, have been reported by one laboratory to be expressed during latency (21). Transcripts for VZV ORF4, ORF21, ORF29, ORF62, and ORF63 have also been detected during latency in rodent models of infection (2, 18, 31).

VZV ORF21 encodes a 115-kDa protein located in both the nucleus and the cytoplasm of virus-infected cells (22). The protein is present in the nucleocapsid but is probably not associated with the tegument. ORF21 protein does not transactivate putative immediate-early, early, or late VZV promoters (40), but immediate-early ORF62 protein transactivates the ORF21 promoter (7, 40). ORF21 is expressed as a 3.1- to 3.5-kb transcript in cell culture, and the 5′ and 3′ boundaries of the transcript, as well as its promoter, have been mapped (6, 7, 15, 40).

VZV ORF21 has homologs in each of the alphaherpesviruses, including herpes simplex virus type 1 (HSV-1) (35), HSV-2 (38), simian varicella virus (5), and pseudorabies virus (PRV) (19). VZV ORF21 shares 27% identity with its HSV-1 homolog, UL37. UL37 protein is a phosphoprotein that is expressed shortly before viral replication has been initiated (1, 35). Like VZV ORF21 protein, HSV UL37 protein localizes to the nucleus and cytoplasm (25, 35). However, unlike VZV ORF21 protein, HSV UL37 protein is a tegument protein (24, 33). HSV UL37 protein shuttles between the nucleus and the cytoplasm (38) and adheres to single-stranded DNA columns in the presence of HSV ICP8 (34). VZV ORF21 protein does not form a DNA binding complex with ORF29 protein (the homolog of HSV ICP8) (9).

Since VZV ORF21 is expressed during latency, we asked whether the gene is required for virus replication in vitro and for the establishment of latent infection. Furthermore, since HSV-1 UL37 shares some but not all of the properties of VZV ORF21, we tested whether UL37 could complement ORF21 in vitro. Here we show that ORF21 is essential for VZV replication in cell culture but not for latent infection and that HSV-1 UL37 cannot substitute for ORF21 during VZV replication in vitro.

MATERIALS AND METHODS

Cells and viruses. Human melanoma (MeWo) cells were used for virus propagation and transfection. Recombinant VZV was derived by using cosmids from the attenuated Oka strain of VZV. A MeWo cell line expressing VZV ORF21 was constructed by inserting a KpnI-BsrGI fragment (VZV nucleotides 30756 to 33865 [11]) which contains ORF21 from plasmid pGORF21A (40) into the KpnI and XhoI sites of pcDNA3 (Invitrogen, Carlsbad, Calif.). During the plasmid construction, the BsrGI site from VZV ORF21 and the XhoI site from pcDNA3 were blunted with the Klenow fragment of DNA polymerase I. The resulting plasmid, pCMV-ORF21, contains VZV ORF21 driven by the human cytomegalovirus immediate-early promoter and the G418 resistance gene driven by the simian virus 40 early promoter. Plasmid pCMV-ORF21 was linearized with PvuI, and melanoma cells were transfected with the plasmid by the calcium phosphate procedure. The cells were treated with trypsin after 2 days and plated onto 100-mm-diameter dishes, and the next day G418 (Invitrogen) was added at a concentration of 400 μg/ml. After 7 days, the concentration of G418 was reduced to 250 μg/ml and individual colonies were isolated and amplified. A cell clone containing ORF21 DNA was verified by PCR.

Cosmids and transfections. VZV cosmids NotI A, NotI BD, MstII A, and MstII B contain the entire genome of the Oka strain of VZV (Fig. 1) and can recombine after transfection of cells to generate recombinant virus. VZV ORF21
FIG. 1. Construction of recombinant VZV with deletions in ORF21 or with an insertion of HSV-1 UL37. The VZV genome is 124,884 bp in length (line 1) and consists of unique long (UL), unique short (US), internal repeat (IR), and terminal repeat (TR) regions (line 2). Transfection of cells with four cosmids spanning the VZV genome (lines 3 and 4) results in infectious virus. Cosmid NotIA-21D (line 5) has a deletion of codons 1 to 1030 of ORF21 (with the last eight codons lacking a start codon), while in cosmid NotIA21d (line 6), codons 89 to 1030 of ORF21 are deleted. Cosmid MstII A-UL37 (line 7) has HSV-1 UL37, driven by its own promoter, inserted between VZV ORF65 and ORF66.

is located between nucleotides 30759 and 33872 of the viral genome (11). To construct a cosmid with the ORF21 deletion, VZV cosmid NotI A was digested with HpaII by using the RecA-assisted restriction endonuclease cleavage procedure (14). Two single-stranded 40-base oligonucleotides, TGGATAGAGATCGTTTGAAGAGATCGGAATTC, and ATGTGCTCTGTGCGTGAACCAGGGATACAGTGGGAGTGA, centered around HpaII sites at VZV nucleotides 30700 and 33847 (11) were annealed to cosmid NotI A with Escherichia coli RecA. HpaII methylase was added to methylate the remaining HpaII sites, the DNA-RecA complexes were dissociated by heating to 65°C, and the DNA was digested with HpaII, releasing a 3,148-bp fragment between the two unmethylated HpaII sites. The large DNA fragment, lacking 99% of ORF21, was ligated to itself, and VZV cosmid NotIA-21D was selected. This cosmid has a deletion beginning 60 bp upstream from the start codon of ORF21 and ending 26 bp upstream of the stop codon of ORF21 (Fig. 1).

To create a cosmid with a slightly smaller deletion in ORF21, two single-stranded 40-base oligonucleotides, AGTTTGCTCTTGGCCTACGTTAATTTTGCGCCCTGTGA and ATGGTTGCTCTTGGCCTACGTTAATTTTGCGCCCTGTGA, centered around HpaII sites at VZV nucleotides 31021 and 33847 (11) were annealed to cosmid NotI A with Escherichia coli RecA and the remainder of the RecA-assisted restriction endonuclease procedure was performed as described above. The DNA was then digested with HpaII, releasing a 2,827-bp fragment between the two unmethylated HpaII sites, and the large DNA fragment was ligated to itself to create VZV cosmid NotIA21d. This cosmid, which lacks 90% of ORF21, has a deletion beginning 263 bp downstream from the start codon of ORF21 and ending 26 bp upstream of the stop codon of ORF21 (Fig. 1).

To construct a VZV cosmid that contains HSV-1 UL37, a 4.6-kb AphiII-NdeI fragment containing UL37 and its promoter (29) and poly(A) site (HSV-1 nucleotides 80467 to 85061) was isolated from plasmid HSV-1 Xhol E (3). The DNA was blunted with the large (Klenow) fragment of DNA polymerase I and inserted into the AsI site (VZV nucleotide 112853) of VZV cosmid MstII A that had been blunted with Klenow fragment. Two independent clones of the cosmid, MstII A-UL37A and MstII A-UL37B, were constructed.

Melanoma cells or cells stably transfected with plasmid pCMV-ORF21 were used for cosmids transfections. Cosmids were linearized using NotI or BsrGI and transfected into melanoma cells or the ORF21 cell line. Cells were treated with trypsin and replated in 100-mm diameter dishes each week until a cytopathic effect (CPE) was detected.

Plasmid pBORF21, containing ORF21 with additional flanking sequences, was used to rescue the deletion in ORF21. Plasmid pBORF21 was digested with AseI, and a 4-kb fragment containing ORF21 was isolated. Melanoma cells were transfected with 1 μg of the DNA fragment containing ORF21, 50 ng of pCMV62, and 0.5 μg of virion DNA from the ORF21 large-deletion mutant. When CPE was detected, cell-free virus was prepared by sonication (36) and cells and media were infected with serial dilutions of virus. Cells with CPE from the highest dilution were used for two more rounds of purification by serial dilution of cell-free virus.

Northern blotting, primer extension, Southern blotting, and immunoblotting. Total cell RNA was prepared from the cell line infected with recombinant VZV by using an RNAeasy mini kit (Qiagen, Santa Clarita, Calif.). Northern blotting was performed with 20 μg of the total RNA per lane, and hybridizations were performed as previously described using oligonucleotide probes (40).

Oligonucleotide ORF20-P1 (CCACATACGGTTTGTTCGTTT), corresponding to VZV nucleotides 30420 to 30440 and located 45 bp downstream from the ORF20 start codon, was used for both Northern blotting and primer extension as previously described (40). A 1.97-kb HinIII fragment derived from plasmid pCMV62 and containing a portion of ORF62 was also used to generate a probe for Northern blotting.

Southern blotting was performed with VZV DNAs purified from nucleocapsids. DNA was cut with BamHI, EcoRI, or BglII, fractionated on 0.8% agarose gels, transferred to nylon membranes, and probed with a [32P]dCTP-labeled 4.0-kb AseI fragment (VZV nucleotides 30211 to 34225) which contains ORF21.
a 4.6-kb fragment (HSV-1 nucleotides 80467 to 85061) that includes a portion of UL37, or all four VZV cosmid DNAs.

Immuneblotting was performed with lysates from cells infected with VZV recombinants expressing HSV-1 UL37. The blots were incubated with rabbit antibody to HSV-1 UL37 protein (a kind gift from Frank Jenkins) or murine monoclonal antibody to VZV glycoprotein E (e; Chemicon, Temecula, Calif.) followed by horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibody. The blots were then developed by using enhanced chemiluminescence (Supersignal; Pierce Chemical Company, Rockford, Ill.).

Reverse transcriptase PCR. Cells were infected with cell-free VZV, and RNA was isolated and treated with RNase-free cDNA was prepared with 1 μg of RNA, 50 U of Superscript II reverse transcriptase (Invitrogen), and 0.5 μg of oligo(dT)12-18 for 50 min at 42°C. PCR was performed with oligonucleotides AAA ATGATCTACTGGGATAC and ACTACCCGCATATTGCTGCG, corresponding to VZV nucleotides 117287 to 117307 and 117117 to 117137, and the reaction products were run on an agarose gel.

Growth studies of recombinant VZV. Cells containing VZV mutants were serially diluted and used to infect melanoma cells or the cell line expressing ORF21. Plaque diameters were measured microscopically. The mean size of 20 plaques was determined, and the r test was used to determine whether the differences were significant.

Cells infected with about 200 PFU of VZV ORF21 mutants were used to infect melanoma cells. At 1, 2, 3, 4, and 5 days after infection, the cells were treated with trypsin and serial dilutions were used to infect melanoma cells. The cells were fixed and stained with crystal violet 1 week after infection, and the number of plaques was determined.

Animal experiments. Four- to five-week-old female cotton rats were inoculated intramuscularly at six sites on each side of the thoracic and lumbar spine, with uninfected or VZV-infected melanoma cells containing 1.5 × 10⁵ or 3 × 10⁵ pfu of recombinant virus in a volume of 50 μL. One month after inoculation, the animals were sacrificed and the DNA from thoracic and lumbar dorsal root ganglia was pooled. PCR was performed using 500 ng of cotton rat DNA and primers for ORF63 as previously described (32). Serial dilutions of VZV cosmids NotI A DNA, digested in 500 ng of genomic DNA from uninfected cotton rat dorsal root ganglia DNA, were used to estimate the copy number of latent viral genomes. The PCR products were run on agarose gels, blotted onto nylon membranes, and hybridized with a [32P]dCTP-labeled probe to ORF63. Copy numbers were estimated by densitometry by use of a phosphorimager. The lower limit of detection by this assay was 10 copies of VZV DNA mixed with 500 ng of DNA from dorsal root ganglia of uninfected animals.

Total RNA was isolated from infected and infected cotton rat dorsal root ganglia. RNA from VZV-infected melanoma cells served as a positive control. Ganglia RNA from each animal was pooled and treated with RNase-free DNase I, and cDNA was prepared as described above. PCR was performed using the cDNA with primers for ORF63 as described above. The PCR products were hybridized to a radiolabeled ORF63 probe.

RESULTS

VZV ORF21 is essential for the replication of virus in cell culture. To produce a virus that does not express VZV ORF21 protein, cosmids NotI A-21D, which has a deletion covering 99% of ORF21, was constructed (Fig. 1). Transfection of melanoma cells with VZV cosmids NotI A-21D, NotI BD, MstII A, and MstII B failed to generate infectious virus, although transfections were performed multiple times and the transfected cells were passaged for more than 12 weeks. In contrast, transfection of melanoma cells with cosmids NotI A, NotI BD, MstII A, and MstII B, which contain the entire VZV genome, including ORF21, consistently yielded infectious VZV, with CPE detected by 8 days after transfection.

Therefore, a cell line expressing ORF21 from the cytomegalovirus promoter was constructed in melanoma cells to complement the VZV mutant with the ORF21 deletion. Transfection of cosmids NotI A-21D, NotI BD, MstII A, and MstII B into an ORF21-expressing cell line yielded infectious virus. CPE was observed 33 days after transfection. The resulting virus was termed VZV ROka21D. Further experiments showed that VZV ROka21D could be propagated in the ORF21 cell line but not in control melanoma cells. Similarly, transfection of melanoma cells with cosmids NotI A-21D (which has a slightly smaller deletion in ORF21), NotI BD, MstII A, and MstII B failed to yield virus, but transfection of these cosmids into the ORF21-expressing cell line did result in infectious virus. These findings indicate that ORF21 is essential for the replication of VZV in cell culture.

Southern blotting was performed to verify that VZV ROka21D had the expected genome structure. Digestion with BamHI of viral DNA from the parental virus, VZV ROka, showed 9.6- and 2.9-kb fragments, and digestion of ROka21D DNA yielded only a 9.3-kb fragment due to the 3.1-kb deletion in ORF21 (Fig. 2A). EcoRI digestion showed 9.1- and 4.7-kb fragments with VZV ROka DNA and a 10.6-kb fragment with VZV ROka21D DNA. BglII digestion showed 19.7-, 5.8-, and 2.0-kb fragments with VZV ROka DNA and a 24.3-kb fragment with VZV ROka21D DNA. Thus, VZV ROka21D contains the expected deletion in ORF21.

Rescue of the deletion in VZV ORF21 allows the virus to replicate in cell culture. Transfection of melanoma cells with VZV cosmids NotI A-21D, NotI BD, MstII A, MstII B, and a DNA fragment containing ORF21 resulted in CPE 8 days after transfection. The resulting virus was plaque purified by three rounds of limiting dilution and termed ROka21DR. To verify that this virus had ORF21 restored, Southern blotting was performed with ROka21DR DNA and ROka DNA. Digestion of both DNAs with BglII followed by hybridization with an ORF21 probe showed identical bands of 19.7, 5.8, and 2.0 kb (Fig. 2B). Thus, the deletion in ORF21 was restored in ROka21DR.

Transcription of ORF20 is not impaired in cells infected with VZV lacking ORF21. VZV ORF21 and ORF20 are transcribed in opposite directions, and the two genes share a 284-bp region, located between them (Fig. 3A). To determine the precise transcription initiation site of ORF20, primer extension analysis was performed with an ORF20-specific primer (ORF20-P1) which anneals at VZV nucleotides 30420 to 30440, located 45 bp downstream of the ORF20 start codon. A cluster of VZV-specific primer extension products was detected with 5′ ends corresponding to VZV nucleotides 30550, 30549, and 30547 (Fig. 3B). These products begin 75, 74, and 72 bp upstream of the start codon for ORF20. Two putative TATA boxes are present 6 and 26 nucleotides upstream of the ORF20 transcriptional start site. A CAAT sequence is located 66 bp upstream from the transcription initiation site of ORF20, which is 84 bp from the upstream boundary of the ORF21 gene deletion.

To verify that the transcription of ORF20 was not impaired by the deletion of ORF21 in ROka21DA, Northern blotting was performed with RNA from VZV ROka- and ROka21D-infected cells that was hybridized with ORF20- and ORF62-specific probes. A 1.6-kb ORF20 transcript and a 4.3-kb ORF62 transcript were detected in cells infected with ROka and ROka21D (Fig. 4). The two VZV transcripts from ROka-infected cells were more abundant than the transcripts from ROka21D-infected cells, indicating a higher level of infection in this experiment in cells infected with VZV ROka than in those infected with ROka21D. To determine whether the transcription of ORF20 was impaired in cells infected with...
ROka21D, the ratio of the levels of ORF20 and ORF62 transcripts in ROka-infected cells to those in ROka21D-infected cells was determined. The ratio of the density of the ORF20 bands for ROka-infected cells to that of the ORF20 band for ROka21D-infected cells was 5.1:1, and the ratio of the density of the ORF62 band for ROka-infected cells to that of the ORF62 band for ROka21D-infected cells was 5.2:1. These results indicate that the transcription of ORF20 is not impaired by the deletion in ROka21D.

**HSV-1 UL37 does not complement VZV ORF21.** To determine whether HSV-1 UL37, the homolog of VZV ORF21, can complement the replication defect in ROka21D, the gene encoding HSV-1 UL37 protein was inserted into a VZV cosmid. Two independent cosmid clones, MstII A-UL37A and MstII A-UL37B, that contain the HSV-1 UL37 gene driven by its own promoter were constructed. Melanoma cells were transfected with VZV cosmids NotI A, NotI B, MstII B, and MstII A-UL37B. CPE was detected 9 days after transfection, and the resulting viruses were termed ROkaUL37A and ROkaUL37B.

To determine if HSV-1 UL37 can substitute for VZV ORF21, melanoma cells were transfected with VZV cosmids NotIA-21D, NotI B, MstII B, and MstII A-UL37A or MstII A-UL37B. CPE was detected 9 days after transfection, and the resulting viruses were termed ROkaUL37A and ROkaUL37B.

To verify that HSV-1 UL37 was present in the recombinant VZV, Southern blotting was performed with a UL37 probe. Digestion of VZV ROkaUL37A and ROka21D/UL37A DNA with EcoRI yielded a 21.6-kb band with the UL37 probe (Fig. 2C).

To ascertain that HSV-1 UL37 was expressed in the appropriate viruses, immunoblotting was performed with rabbit antibody to the protein. Cells infected with VZV ROkaUL37A, ROka21D/UL37A, and HSV-1 expressed a protein of 120 kDa that was detected with the antibody to HSV-1 UL37 protein, while cells infected with VZV ROka did not express this protein (Fig. 5A). To verify infection, immunoblots were also incubated with antibody to VZV gE (Fig. 5B).

**Growth characteristics of VZV ORF21 deletion mutants in cell culture.** The ORF21-expressing cell line was infected with cells containing VZV ROka or ROka21D, and plaque sizes were determined 7 days later. The mean length and width of plaques (± standard deviations) after infection with ROka21D were 0.3 ± 0.1 and 0.2 ± 0.1 mm, respectively, while the mean length and width of plaques after infection with ROka were 0.8 ± 0.1 and 0.6 ± 0.1 mm, respectively. Thus, the mean plaque size for ORF21 deletion mutants was significantly smaller ($P < 0.01$) than the plaque size for parental virus.

To study the growth of the various recombinant viruses with VZV ORF21 deleted and/or expressing HSV-1 UL37, viral titers were measured during a 5-day growth analysis. VZV ROka, ROka21DR, ROkaUL37A, and ROkaUL37B grew to similar titers in melanoma cells (Fig. 6). In contrast, VZV ROka21D and ROka21D/UL37A were unable to replicate in cell culture and no virus was detectable after the second day of infection.

To determine if a late VZV gene could be expressed in cells...
infected with the ORF21 deletion mutant, cell-free VZV ROka and ROka21D were prepared and used to infect melanoma cells. One day after infection, gE was detected by immunofluorescent staining in cells infected with VZV ROka or ROka21D but not in uninfected cells (data not shown). To further verify that gE was expressed, melanoma cells were infected with cell-free virus, RNA was isolated 2 days later, and reverse transcription was performed. The resulting cDNA was amplified by PCR with primers for gE, and gel electrophoresis was performed. A 190-bp band corresponding to the amplified portion of gE cDNA was detected in cells infected with VZV ROka or ROka21D but not in uninfected cells or from reactions in which reverse transcriptase was not used (Fig. 7). Thus, VZV ORF21 is not essential for the expression of VZV gE.

**VZV ORF21 is not required for establishment of latency.** To determine if VZV ORF21 is important for the virus to initiate a latent infection, cotton rats were inoculated paraspinally with VZV ROka and ROka21D. In the first set of experiments, the animals were inoculated with $1.5 \times 10^5$ PFU of virus, and in the second experiment they received $3 \times 10^5$ PFU of virus. One month after inoculation, the animals were sacrificed, the thoracic and lumbar dorsal root ganglia were removed, and DNA and RNA were isolated. PCR of DNA from dorsal root ganglia for VZV ORF63 followed by Southern blotting showed that viral DNA was detected ($\geq 10$ copies per 500 ng of ganglia DNA) in ganglia from 12 of 20 animals inoculated with VZV ROka and from 7 of 21 animals inoculated with ROka21D (Fig. 8). The geometric mean numbers of VZV genome copies from PCR-positive ganglia per 500 ng of ganglia DNA were similar for animals infected with ROka (37 copies) and for those infected with ROka21D (54 copies).

To verify that the animals developed latent infection, cDNA was prepared from ganglia RNA and PCR was performed with primers specific for VZV ORF63, followed by Southern blotting. ORF63 is expressed during latency in humans and rodents (8, 18, 31). ORF63 RNA was detected in 10 of 19 animals inoculated with VZV ROka and in 11 of 21 animals inoculated with ROka21D (Fig. 9 and data not shown).

**DISCUSSION**

We have shown that VZV ORF21, a gene expressed during latency, is essential for replication in cell culture but is not required for establishment of latent infection. Infectious virus is required for establishment of latency, since a prior study showed that inoculation of animals with heat-inactivated virus did not result in latent infection (32). Here we show that replication-competent VZV is not required for latency.

The homolog of ORF21 in HSV-1 is UL37, which like ORF21 is essential for virus replication (12), while its homolog in PRV, also designated UL37, is dispensable (19). The deletion of HSV-1 or PRV UL37 results in reduced reenvelopment
of virions during fusion with the nuclear membrane, with accumulation of immature virions. Comparison of the amino acid sequences of VZV ORF21, HSV-1 UL37, and PRV UL37 proteins shows that ORF21 protein is slightly more homologous to PRV UL37 protein than to the HSV-1 protein. VZV ORF21 shows 29% identity with its PRV homolog and 27% identity with its HSV-1 homolog. However, since VZV ORF21 and HSV-1 UL37 are both essential for virus replication, the function of the VZV protein may more closely resemble that of its homolog in HSV-1 than that of its homolog in PRV.

HSV-1 UL37 was unable to complement the activity of VZV ORF21. While some VZV genes (ORF51 [4], ORF62 [13], and ORF61 [27]) can substitute for their HSV homologs, other VZV genes (ORF4 [28] and ORF29 [39]) cannot complement their HSV counterparts. VZV ORF21 and HSV-1 UL37 proteins show homology throughout the length of their sequences except for their amino termini. The regions of the two proteins that show the highest amino acid identity (VZV ORF21 protein, amino acids 227 to 235; HSV-1 UL37 protein, amino acids 260 to 268) include a portion of the leucine-rich region, which

FIG. 4. Transcription of VZV ORF20 and ORF62 in cells infected with VZV strains ROka and ROka21D. The total RNA from uninfected or infected cells was hybridized with a radiolabeled ORF20 or ORF62 probe on a Northern blot. A 1.6-kb band corresponding to ORF20 is present, as is a 4.3-kb band representing ORF62.

FIG. 5. Immunoblot showing HSV-1 UL37 expression in cells infected with recombinant VZV. (A) A 120-kDa protein (indicated by an arrow) is present in lysates of cells infected with HSV-1, ROkaUL37A, or ROka21D/UL37A incubated with antibody to HSV-1 UL37 protein. (B) Bands of 90 to 100 kDa that react with antibody to VZV gE are present in cells infected with VZV ROka or in cells infected with VZV expressing UL37 but not in cells infected with HSV-1.

FIG. 6. Growth of parental VZV and ORF21 deletion mutants in melanoma cells. Cells were infected with the indicated viruses, and each day after infection the cells were treated with trypsin and the virus titers were determined.

FIG. 7. gE mRNA is expressed in melanoma cells infected with an ORF21 deletion mutant. RNA was isolated from infected cells, cDNA was prepared in the presence (+) or absence (−) of reverse transcriptase (RT). Molecular sizes (in base pairs) of DNAs are indicated on the left.
has been shown to act as a nuclear export signal in HSV-2 UL37 (38). Other functional domains of these proteins have not been determined.

VZV ORF21 was not required for the expression of VZV gE. The deletion of other essential herpesvirus genes, such as HSV UL5 and UL29 (10), still allows the expression of viral glycoproteins in virus-infected cells. HSV mutants lacking these genes have been tested in animals as candidate vaccine viruses (10). Similarly, ROka21D may be considered a candidate vaccine virus, since it cannot replicate but still expresses viruses (10). Similarly, ROka21D may be considered a candidate vaccine virus, since it cannot replicate but still expresses VZV gE and probably other late gene products. ROka21D can establish a latent infection but should not be able to reactivate.

VZV ORF21 is the first VZV gene expressed during latency that has been shown to be dispensable for the establishment of latent infection. VZV is different from the other alphaherpesviruses in which latency-associated transcripts (LATs) are the only transcripts that are efficiently transcribed during latency. While some studies indicate that the deletion of the HSV-1 LAT does not reduce the level of latent HSV-1 DNA (16, 20), other studies show a reduction in latent viral DNA. For example, inactivation of the HSV LAT reduced the number of latently infected neurons in mice about threefold in one study but did not affect the HSV-1 genome copy number of individual infected neurons (37). Similarly, the deletion of the LAT resulted in a two- to threefold reduction in the number of latently infected neurons in rabbits (30). We did not detect a reduction in the amount of latent VZV DNA in ganglia from virus with the ORF21 deletion compared to that from the parental virus. Since ORF21 RNA has been detected in both human (6, 17) and rodent (31) ganglia latently infected with VZV, the gene may have an important role in another aspect of latency. While the patterns of expression of other latent VZV genes are similar in humans and rodents (2, 6, 17, 18, 26, 31), there are differences between infection in rodents and infection in humans. VZV does not cause disease in rodents, and reactivation of latent VZV has not been demonstrated. Future studies will focus on developing animal models of VZV reactivation from latency to determine the role of ORF21 in reactivation.

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REFERENCES


