JOURNAL OF VIROLOGY, July 1995, p. 4274–4282 Vol. 69, No. 7
0022-538X/95/$04.00+0

The Transcriptional Regulatory Proteins Encoded by Varicella-Zoster Virus Open Reading Frames (ORFs) 4 and 63, but Not ORF 61, Are Associated with Purified Virus Particles

PAUL R. KINCHINGTON,[1,2]* DANA BOOKEY,[1] AND STEPHANIE E. TURSE[1]

Departments of Ophthalmology[1] and of Molecular Genetics and Biochemistry,[2] University of Pittsburgh, Pittsburgh, Pennsylvania 15213

Received 12 January 1995/Accepted 7 April 1995

Of the five varicella-zoster virus (VZV) open reading frames (ORFs) known to encode proteins which influence viral transcriptional events, two (ORFs 10 and 62) encode proteins associated with the tegument of virus particles, where they may function during the immediate-early events of infection. In this study, antibodies which recognize the products of the three additional VZV ORFs, ORFs 4, 61, and 63, were made and used to characterize their association with virus particles. ORF 4 encoded a 52-kDa polypeptide, and antibodies to ORF 63 reacted with polypeptides of 47 and 28 kDa. Antibodies to ORF 61 recognized heterogeneous polypeptides of 62 to 66 kDa in cells infected with a vaccinia virus recombinant expressing ORF 61 and in VZV-infected melanoma cells but reacted very weakly with polypeptides of VZV-infected human foreskin fibroblasts, suggesting that cell-specific factors were involved in ORF 61 protein accumulation. Analysis of virus particles purified from melanoma cells indicated that a 52-kDa polypeptide from ORF 4 and the 47-kDa polypeptide from ORF 63, but not any from ORF 61, were associated with virus particles. The virion proteins were likely components of the tegument, as they were not solubilized by treatment of virus with mild detergents and were completely resistant to trypsin digestion unless prior envelope solubilization was performed. The products of ORFs 4 and 63 were not found in purified VZV nucleocapsids. These results suggest that forms of the ORF 4- and ORF 63-encoded transcriptional regulatory proteins are also structural and may also have roles in the immediate-early events of infection.

Varicella-zoster virus (VZV), a ubiquitous human herpesvirus, causes two clinically distinct human diseases, varicella (chickenpox) and, after a long period of viral latency in the sensory ganglia, zoster (shingles). Despite very efficient pathogenicity and spreading in a susceptible population, the growth of the virus outside the human host is considerably limited, and as a consequence, its biology has been difficult to study. However, comparison of the VZV genome sequence (6, 7) with that of the better-characterized herpes simplex virus type 1 (HSV-1) (32) has shown that the two viruses are closely related. Most VZV open reading frames (ORFs) encode products with amino acid homologies to characterized HSV-1 proteins, and the respective genes are largely colinear on the viral genomes (6). As a result, the functions of many putative VZV proteins have been predicted from their HSV-1 homologs (7). However, characterization of the encoded VZV proteins has revealed aspects unique to VZV, particularly concerning virus structural proteins (5, 24) and proteins with functions involved in the regulation of viral transcription (8, 22, 23, 38, 41, 44).

The difficulty in obtaining high titers of cell-free VZV from culture and conducting high-multiplicity infections has made it hard to resolve VZV transcriptional events and determine how they are regulated. However, evidence suggests that transcription occurs in a temporal, controlled fashion (50) similar to that originally characterized for HSV-1, such that each viral gene can be categorized into one of three groups (immediate-early [IE], early, and late), depending on the requirements for transcription (19, 20). IE genes are transcribed in the absence of de novo viral protein synthesis (19). Several VZV IE regulated proteins have been reported (30, 53), of which one has been shown to be a 175- to 180-kDa phosphoprotein encoded by VZV ORF 62 (14, 24). The ORF 62 polypeptide shows considerable homology, both at the amino acid and functional levels, to the major HSV-1 IE transcriptional regulatory protein ICP4 (9, 10, 12, 13). VZV ORFs 4, 61, and 63 also encode proteins which are functional or positional homologs of the HSV-1 IE gene products ICP27, ICP0, and ICP22, respectively (6, 36, 38, 44). However, the regulation of these genes remains to be defined.

Several herpesviruses have been shown to encode bifunctional structural proteins which act upon infection to stimulate IE gene transcription. The best characterized is the HSV-1 α *trans*-inducing factor (αTIF, VP16, or Vmw65; reviewed in reference 49), an abundant virion tegument protein which complexes with cellular transcription factors upon infection (3, 15, 29, 35, 43, 48) and activates IE gene transcription through specific DNA elements present in the IE gene promoters known as TAATGARAT motifs (31, 47). Additional HSV-1 structural proteins, including the products of UL46 and UL47 (62, 63) and possibly low-abundancy structural forms of the HSV-1 IE proteins ICP4 and ICP0 (60, 61), may play accessory roles. VZV ORF 10 encodes a virion transcriptional transactivator (24, 37) with considerable homology to HSV-1 αTIF (6, 7). However, unlike HSV-1 αTIF, which is essential for virus assembly (58) and optimal for IE gene transcription (1), VZV ORF 10 can be deleted without affecting viral growth in tissue culture (5). The VZV particle has been shown to contain abundant levels of a 175-kDa tegument polypeptide encoded by ORF 62 (24). As a potent transcriptional activator of many VZV genes (2, 21, 22, 40, 45, 46), the structural form of the ORF 62 product has been speculated to play a role in VZV IE events (24, 28, 40).

* Corresponding author. Mailing address: 1020, Eye & Ear Institute Building, 203 Lothrop St., Pittsburgh, PA 15213. Phone: (412) 647-6319. Fax: (412) 647-5880.

Additional VZV proteins not yet identified may also be bifunctional structural proteins with roles in the IE events in the newly infected cell. VZV ORFs 4, 61, and 63 encode polypeptides which have been shown to influence viral transcription from VZV promoters in transient transfection studies (2, 8, 21–23, 36, 39, 41, 44, 45). ORF 61 products can independently activate transcription as well as effectively repress ORF 62- and ORF 4-mediated transcriptional activation (36, 39, 41, 45). ORF 4 also encodes a transcriptional activator which can function both independently and in conjunction with ORF 62 (8, 21, 38, 44). The function of ORF 63 is less clear, but it has been implicated as a repressor of ORF 62 transcription and may affect transcription of certain VZV early genes (23).

In this study, antibodies specific for the polypeptides of these three VZV ORFs were made, used to identify the encoded products, and used to show that those encoded by ORFs 4 and 63, but not ORF 61, were associated with purified virus particles. As these are proteins with both structural and transcriptional regulatory functions, we postulate that they may also play roles in the IE events of infection.

MATERIALS AND METHODS

Cells and virus. VZV strain Scott (isolate 71004) has been described previously (27) and was used throughout these studies at less than 12 passages beyond its original isolation. Virus was grown at 35°C on human foreskin fibroblasts (HFF cells; line 521, obtained from M. Vincent, Uniformed Services University of the Health Sciences, Bethesda, Md.) or on a human melanoma cell line (MeWo cells; kindly supplied by C. Grose, University of Iowa, Iowa City), as previously described (56). The parental vaccinia virus, WR (originally obtained from B. Moss, National Institute of Allergy and Infectious Diseases, Bethesda, Md.), and vaccinia virus recombinants were grown on Vero and human TK$^-$143 cells as described previously (4). All cells were maintained at 37°C in Eagle's minimal essential medium supplemented with 4% Serum Plus (Hazleton Biologics Inc., Lenexa, Kans.), 4% fetal bovine serum, and an antibiotic mixture of 100 U of penicillin per ml and 0.1 mg of streptomycin per ml.

Antibodies. Antibodies which recognize the products of ORFs 4, 61, and 63 have been described previously (42). These were generated against VZV ORF protein fusions to the maltose binding protein (MBP) by using the pMALC Fusion Protein Expression System (New England Biolabs Inc., Beverly, Mass.). Antibodies to two ORF 4 fusion proteins were used in these studies. The first antibody was made against a fusion of MBP to the C-terminal 330 amino acids of the 452-amino-acid ORF 4 product, expressed from a pMalC clone containing VZV sequences from a *Bgl*II site at position 4377 within ORF 4 to a *Pvu*II site downstream of the ORF at position 2036 (coordinates are given in base pairs with respect to the published VZV sequence [7]). The second antibody was made against a complete ORF 4-MBP fusion, expressed from a pMalC clone of VZV sequences from an *Nco*I site spanning the ORF 4 ATG (at position 4141) to the *Pvu*II site at position 2036. A complete ORF 61-MBP fusion protein was expressed from a pMalC clone of VZV DNA from the *Nco*I site spanning the ATG (at position 104,484) to an *Acc*I site downstream of the ORF (at position 102,708), and a complete ORF 63 fusion protein was made from an in-frame clone of an *Sty*I-*Bam*HI fragment containing the ORF, representing VZV sequences from positions 110,573 to 111,577. All fusion proteins were expressed in *Escherichia coli* DH5α containing the plasmid clones and were purified from bacterial sonicates by amylose affinity chromatography, as recommended by the manufacturer of the protein expression kit. The protease inhibitors *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK) and *N*-α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK) were added at 100 μM to all solutions to inhibit protease activity. Antibodies to both native soluble protein and fusion protein presented in unfixed sodium dodecyl sulfate (SDS)-polyacrylamide gel fragments were made in rabbits simultaneously, using Freund's adjuvants. Rabbit antibodies to peptides derived from ORFs 29, 10, and 62 have been described previously (24, 25). Antibodies to the product of ORF 14 (gpV) were made by vaccination of rabbits with a live vaccinia virus recombinant expressing this ORF (26).

Vaccinia virus recombinants. Vaccinia virus recombinants expressing ORFs 4, 61, and 63 were constructed by using the vector pSC11 (4). DNA fragments containing each complete VZV ORF were prepared by using the restriction enzyme sites described for the preparation of complete ORF fusion clones and cloned into the unique *Sma*I site of pSC11. Clones containing the ORFs in the correct and incorrect orientations with respect to the vaccinia virus p7.5 promoter were identified, and the plasmid DNA was transfected into Vero cells infected with the parental vaccinia virus WR. Resulting recombinant thymidine kinase-negative (TK$^-$) viruses were selected by growth on TK$^-$143 cells in the presence of 50 μg of bromodeoxyuridine per ml. As pSC11 also contains the β-galactosidase gene under control of the vaccinia virus promoter p11, additional identification of recombinants was achieved by incorporating 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) at 300 μg/ml in an agarose overlay at 2 days postinfection, as described previously (4).

Radiolabelling of infected-cell polypeptides. VZV-infected MeWo cells were labelled at 12 h postinfection by replacing normal growth media with media containing 20% of the normal concentration of methionine and 30 μCi of [^{35}S]methionine per ml (1,112 Ci/mmol; NEN-Dupont Nemours, Boston, Mass.). Cells were harvested at 72 to 96 h postinfection for virus particle and nucleocapsid preparation.

Virus particle and nucleocapsid purification. Virus particles were purified from VZV-infected MeWo and HFF cells in a manner similar to that described previously (24). VZV-infected cells showing >90% cytopathic effect were harvested, pelleted by low-speed centrifugation, and used to prepare cytoplasmic extracts by Dounce homogenization in serum-free media or phosphate-buffered saline (PBS) at 4°C. In addition, the infected-cell medium was subjected to high-speed centrifugation (40,000 × *g* for 2 h at 4°C) to pellet released material and virus particles (54), which were then combined with the cytoplasmic extracts. Following brief sonication and removal of debris by low-speed centrifugation, the supernatant was centrifuged on 36-ml 5 to 15% Ficoll gradients for 2 h at 25,000 × *g* at 4°C, as described elsewhere (57). A light-diffusing band migrating approximately halfway down the gradient (see Fig. 3a) which contained enveloped virus particles (as determined by electron microscopy) was extracted, diluted in PBS, pelleted by centrifugation at 40,000 × *g* for 2 h at 4°C, and resuspended overnight at 4°C. The preparation was briefly sonicated and then fractionated by centrifugation for 24 h on 30 to 0% reverse glycerol–0 to 50% potassium tartrate gradients made in PBS, as described previously (24). Following concentration by centrifugation, virus was stored at 4°C in PBS containing 10 μM each TLCK and TPCK. Particle purity was monitored by determining loss of radiolabelled uninfected-cell proteins added during cell harvest, by checking of polypeptide content by SDS-PAGE and protein staining, and by testing for lack of the 130-kDa polypeptide from ORF 29 by using antipeptide antibodies specific for this ORF (25).

Nucleocapsids were purified from the nuclei of VZV-infected cells as described by Gibson and Roizman (17), with minor modifications. Briefly, VZV-infected cell nuclei obtained by Dounce homogenization were washed in PBS containing 0.5% Triton X-100 and lysed for 30 min in 50 mM Tris HCl (pH 7.6)–0.15 M NaCl–1% Nonidet P-40–0.5% sodium deoxycholate–50 μg of DNase I per ml at 37°C. Following addition of urea to 0.5 M, residual cell debris was removed by low-speed centrifugation and the supernatant fraction was subjected to sedimentation on two successive 10 to 40% sucrose gradients made in the lysis buffer without DNase I. Two closely sedimenting bands, which were harvested together and concentrated by centrifugation, were observed in the middle of the gradients. Nucleocapsids were resuspended and stored in PBS at 4°C.

Virion localization studies. Virion localization analyses were carried out as described previously (24, 60, 61). For detergent solubilization analyses, preparations of virus particles were treated with PBS containing 10 μM each TLCK and TPCK, with or without 1% Nonidet P-40 and 0.5% sodium deoxycholate, for 10 min at room temperature. Insoluble material was pelleted by centrifugation at 28,000 × *g* for 1 h at 4°C, and equal amounts of soluble and insoluble fractions were subjected to SDS-PAGE for immunoblot analyses. For trypsin protection studies, aliquots of virus particles were treated with trypsin at 0.1 mg/ml (3,055 U/mg, twice crystalized; Gibco BRL Laboratories, Gaithersburg, Md.) in the presence of 100 μM TLCK for 10 min at 37°C, either in the absence or in the presence of 1% Nonidet P-40. The reaction was halted by sequential addition of egg white trypsin inhibitor to 1 mg/ml, TPCK to 100 μM, phenylmethylsulfonyl fluoride to 10 μM, and, finally, 4× SDS-PAGE buffer, after which the mixture was heated to 95°C for 5 min. Polypeptides were electrophoresed on 7.5% bisacrylamide gels and transferred to membranes for immunoreactivity analysis.

Immunoreactivity analyses. Following separation by SDS-PAGE, polypeptides were electrophoretically transferred to Immobilon P membranes (Millipore Corp., Bedford, Mass.) in 192 mM Tris-glycine buffer (pH 8.5) containing 20% methanol. Blocking and all antibody incubations were carried out at room temperature with a buffer containing 25 mM Tris HCl (pH 7.6)–0.15 M NaCl–0.5% Tween 20 (TBS) and 10% nonfat dried milk (TBS-M). Blots were blocked for 1 h prior to incubation with rabbit antibodies at a dilution of 1:100 for 1 h. Excess antibodies were removed by washing, and bound antibodies were detected by using ^{125}I-protein A (NEN-Dupont Nemours) and autoradiography. Levels of bound ^{125}I-protein A were estimated either by scintillation counting or by densitometric analysis of autoradiograms with an Optimus imaging system.

RESULTS

Antibody specificity. To characterize the association of the polypeptides from ORFs 4, 61, and 63 with virus particles, antibodies which specifically recognize them in immunoblot analyses were required. We have recently described the immunoprecipitated polypeptides identified by the antibodies specific for these ORFs, which were made against MBP-VZV ORF fusions as described in Materials and Methods (42). Figure 1 shows the reactivity of these antibodies with immobi-

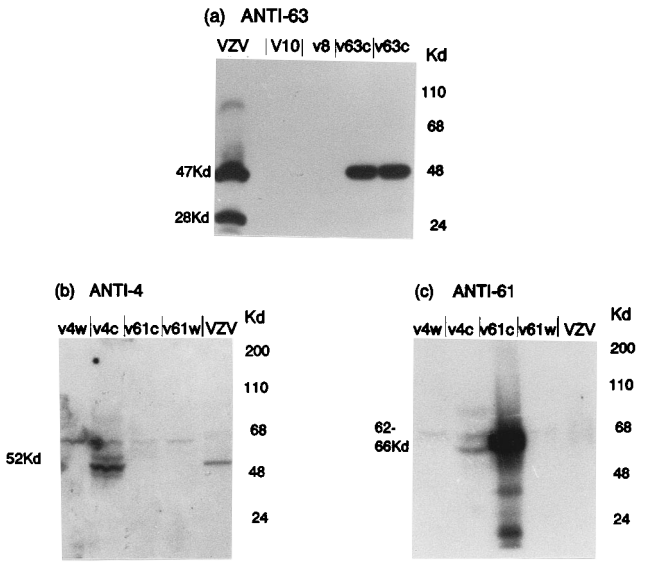

FIG. 1. Immunoreactivities of monospecific antibodies to the fusion protein products of VZV ORF 63 (a), ORF 4 (b), and ORF 61 (c) with polypeptides derived from VZV-infected HFF cells (VZV) or from TK⁻143 cells infected with vaccinia virus recombinants. v63c, v61c, and v4c represent vaccinia viruses expressing the indicated VZV ORF in the correct orientation with respect to the vaccinia virus promoter; v61w and v4w represent vaccinia viruses expressing the ORFs inserted in the incorrect orientation with respect to the vaccinia virus promoter. The lane marked v8 represents vaccinia virus expressing β-galactosidase only, and that marked V10 represents a vaccinia virus expressing VZV ORF 10. The approximate positions of a set of molecular mass markers and their sizes in kilodaltons are shown to the right of each blot, and the sizes of the major polypeptides discussed in the text are shown to the left of each blot.

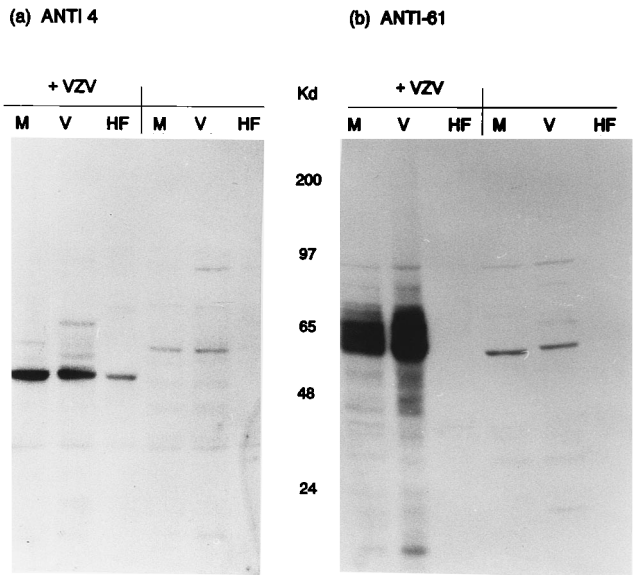

FIG. 2. Immunoreactivities of antibodies to ORF 4 (a) and ORF 61 (b) with identical blots of polypeptides obtained from VZV-infected (+VZV) and uninfected MeWo cells (lanes M), Vero cells (lanes V), and HFF cells (lanes HF). The sizes of the set of molecular mass markers, in kilodaltons, and their approximate positions are indicated between the two blots.

lized SDS-PAGE-separated polypeptides derived from VZV-infected HFF cells and from cells infected with vaccinia virus recombinants expressing each VZV ORF. By expressing the VZV ORFs in vaccinia virus recombinants under control of the powerful vaccinia virus promoter p7.5, we aimed to avoid problems in detection that might arise from weak expression in VZV-infected cells, a strategy which proved important for analysis of ORF 61. Antibodies to ORF 63 (Fig. 1a) identified a 47-kDa polypeptide in extracts of cells infected with VZV and with two independently isolated vaccinia virus recombinants expressing ORF 63 (v63), which was not expressed in cells infected with vaccinia virus controls (V10 and v8). An additional VZV-infected-cell-specific 28-kDa polypeptide was identified; although its origin is uncertain, we suspect that it is a proteolytic degradation product, as its detection varied from preparation to preparation of VZV-infected cells. However, we cannot eliminate the possibility that it reflects a functional truncated form of ORF 63. The 47-kDa polypeptide is larger than the size predicted from its ORF (30,494 Da [7]) but correlates with the immunoprecipitated species previously described (42). When similar analyses were carried out with antibodies made to the C-terminal two-thirds of the ORF 4 product, a VZV-specific 52-kDa polypeptide which was also found in cells infected with the vaccinia virus-ORF 4 recombinant (v4c) but not in cells infected with control vaccinia viruses (v4w, v61w, and v61c) was identified. Antibodies made to the entire ORF 4 fusion showed similar reactivity. The size of the polypeptide is in close agreement with that predicted from its ORF (51,540 Da [7]) and with that of the immunoprecipitated polypeptide (42). Species of 54 and 50 kDa recognized by the ORF 4 antibodies only in the vaccinia virus-ORF 4 recombinant-infected cell extracts were likely the result of vaccinia virus-specific modifications.

Identification of the products of ORF 61 proved not as straightforward. Figure 1c shows a blot identical to that presented in Fig. 1b but probed with ORF 61-specific antibodies. Several polypeptides made in v61c-infected cells were recognized, of which a group of polypeptides of 60 to 66 kDa were predominant (Fig. 1c, v61c). These were not seen in the v61w or v4w controls; the reactivity seen with v4c extracts with 60- and 70-kDa polypeptides was therefore presumed to be nonspecific. During this work, similar studies described by Stevenson et al. (55) identified the products of ORF 61 as 62- to 66-kDa phosphoproteins. However, our antibodies failed to detect polypeptides in VZV-infected HFF cell extracts (the blot presented in Fig. 1c has been overexposed to highlight the lack of reactivity). A second rabbit antibody prepared to ORF 61 yielded similar results (data not shown). This result was important to these studies, as HFF cells have previously been used as the source for the purification of virions (24). As ORF 61 polypeptides can be immunoprecipitated from VZV-infected MeWo cells (42), we compared different cell types infected with VZV under the same conditions. Figure 2 shows immunoblots of polypeptides from equal amounts of MeWo, Vero, and HFF cells, either uninfected or infected with VZV under identical conditions and harvested at 40 h postinfection. When probed with antibodies to ORF 4, all three cell types expressed a VZV-infected-cell-specific 52-kDa polypeptide (Fig. 2a). A difference of approximately fourfold between the amounts of ORF 4 polypeptide present in HFF cells and in MeWo cells (determined by counting of the bound ^{125}I-protein A) may reflect a difference in the number of cells initially infected, as approximately three to five times more MeWo and Vero cells than HFF cells are routinely present at confluence. Probing of an identical blot with anti-ORF 61 antibodies showed that, while 62- to 66-kDa polypeptides were readily detected in VZV-infected Vero and MeWo cells, few were present in VZV-infected HFF cells. From the amount of bound ^{125}I-protein A, the ORF 61 polypeptides in MeWo and HFF cells show greater than 25-fold differential reactivity.

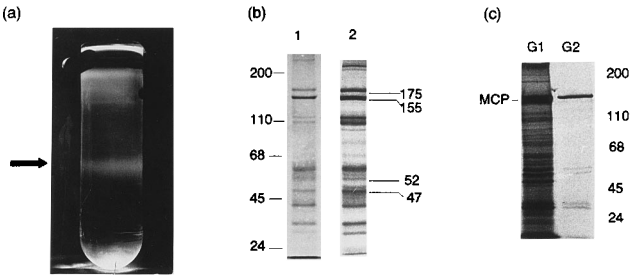

FIG. 3. (a) Photograph of a 5 to 15% Ficoll gradient after centrifugation of VZV-infected cell cytoplasmic extracts as described in Materials and Methods, with illumination from above. The light-scattering band in the middle of the gradient which contained enveloped nucleocapsid-containing virus particles is indicated by an arrow. (b) Electrophoretically separated polypeptides of virus particles after Ficoll and potassium tartrate gradient purification steps, as outlined in Materials and Methods. Polypeptides are shown stained with Coomassie blue R250 (lane 1) or as an autoradiogram of ^{35}S-labelled species (lane 2). The sizes of the molecular mass markers are indicated in kilodaltons, and the 155-kDa major capsid protein (155), the 175-kDa ORF 62 protein (175), and the positions of the 47- and 52-kDa proteins discussed in the text are indicated to the right. (c) Electrophoretically separated [^{35}S]methionine-labelled polypeptides of VZV nucleocapsids after one (G1) and two (G2) sucrose gradient fractionations, as outlined in Materials and Methods. The sizes of marker polypeptides are indicated to the right in kilodaltons, and the major capsid protein (MCP) is also indicated.

HFF cells harvested at later times postinfection showed little more ORF 61 antigen (data not shown). We conclude that analyses of the ORF 61 polypeptides, including the characterization of their association with virus particles, cannot be carried out with VZV-infected HFF cells. VZV-infected MeWo cells were therefore used for the virus particle purification studies.

Virus purification. Procedures for the purification of VZV particles have included use of sucrose gradients, potassium tartrate density gradients (11, 52, 54), and combinations of both (24). The last method yielded over 150-fold purification of virus particles with respect to uninfected cell proteins. Recently, a noninfectious, enveloped nonnucleocapsidated particle which could be separated from virions by using 5 to 15% Ficoll gradients in physiological saline conditions was described for HSV-1 (57). We modified the VZV purification procedure to utilize such gradients for the first fractionation, and Fig. 3a shows a photograph of a Ficoll gradient of centrifuged VZV extracts. A band of light-diffusing material migrating in the middle of the gradient was observed, and this band was found to contain enveloped virus particles by electron microscopy and, when analyzed by SDS-PAGE, abundant levels of the major capsid protein. Concurrent analysis of cytoplasmic extracts of HSV-1 strain 17-infected BHK cells yielded two light-scattering bands characteristic of light and heavy particles, a result which was confirmed by SDS-PAGE (data not shown). Analysis of the gradient fractions predicted to contain light particles indicated that very few light particles were present. However, we cannot eliminate the possibility that the lack stems from a quantitative effect, due to the much lower yields of VZV obtained in comparison with that of HSV-1. Figure 3b shows the stained (lane 1) and [^{35}S]methionine-labelled (lane 2) SDS-PAGE-separated polypeptides of purified VZV preparations following potassium tartrate gradient fractionation. Approximately 25 polypeptide species, of which the most abundant were 155 and 175 kDa, representing the major capsid protein and the product of ORF 62, respectively (24), were identified to be present in the radiolabelled preparations. The protein stain suggests that the majority of cellular proteins have been excluded, as would be expected with purified virus preparations. These preparations also lacked the 130-kDa polypeptides reactive with antibodies directed against ORF 29 products (see below). The virus particles were subsequently examined for reactivity with antibodies to products of ORFs 4, 61, and 63.

Detection of the products of VZV ORFs in VZV particles. Figure 4 shows identical immunoblots of polypeptides obtained from uninfected (lanes 1) and VZV-infected (lanes 2) MeWo cells, alongside preparations of virus particles obtained from MeWo cells (lanes 4), probed with antisera specific for products of ORFs 4, 10, 29, 61, and 63. Antibodies to ORF 29 (nonstructural) and ORF 10 (structural) were included as controls. Equal amounts of VZV-infected and uninfected cell extracts were electrophoresed, with the level of VZV-infected cell extracts being adjusted to give approximately similar levels of bound ^{125}I-protein A as for virus particles when probed with anti-ORF 10 antibodies (Fig. 4b). As expected, antibodies to ORF 10 reacted strongly with a 47-kDa polypeptide present in the virus particle preparation as well as in VZV-infected cell extracts (Fig. 4b). Two forms of ORF 10 which have not been previously described were resolved in the virus particle preparations. While the origin of the two forms is currently unknown, they may represent differentially modified forms incorporated into virions which are not resolved in extracts of VZV-infected MeWo cells (Fig. 4b, lane 2) or in virus from HFF cells (24). In contrast to those to ORF 10, antibodies to ORF 29 reacted with the full-length 130-kDa polypeptide only in

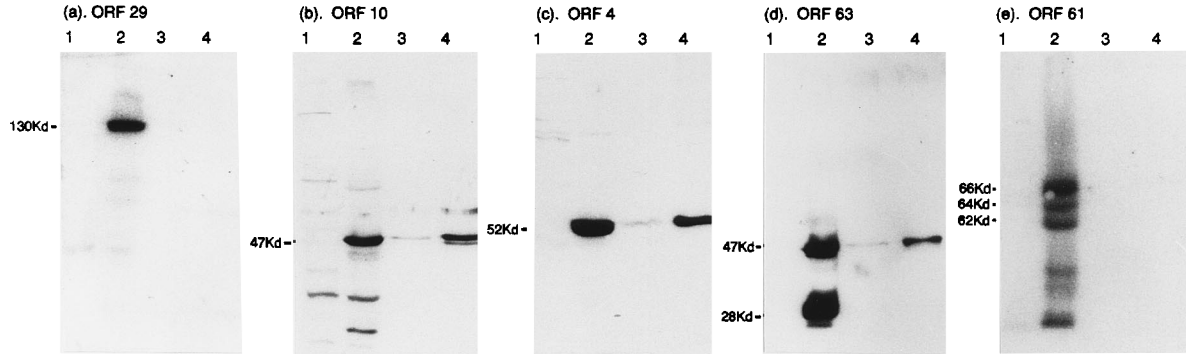

FIG. 4. Immunoreactivities of antibodies to ORF 29 (a), ORF 10 (b), ORF 4 (c), ORF 63 (d), and ORF 61 (e) with identical immunoblots of electrophoretically separated polypeptides obtained from uninfected MeWo cells (lanes 1), VZV-infected MeWo cells (lanes 2), and pelleted and resuspended virus particles (lanes 4). Lanes 3 represent the supernatant of the virus particle suspension after pelleting. The sizes of the specific polypeptides detected by the antibodies are shown to the left of each panel in kilodaltons.

VZV-infected cell extracts (Fig. 4a, lane 2). Very minor species of 50 kDa were detected in lanes 3 and 4 of the gel shown in Fig. 4a, but they likely reflected a nonspecific reaction. When identical blots were reacted with antibodies to the complete ORF 4 (Fig. 4c) and ORF 63 (Fig. 4d) fusion proteins, the respective 52- and 47-kDa polypeptides were found both in virus particles (lanes 4) and in VZV-infected cell extracts (lanes 2). In contrast, polypeptides encoded by ORF 61 were not detected in extracts of VZV particles and were found only in VZV-infected cell extracts (Fig. 4e). These results indicate that the products of ORFs 4 and 63, but not of ORF 61, are associated with virus particles. Interestingly, the 28-kDa polypeptide recognized by the ORF 63 antibodies in VZV-infected cells was not detected in the virus particle extracts, suggesting either that the virion form of the ORF 63 polypeptide is protected from the conditions which generate the 28-kDa form or, if the 28-kDa form is a specific product, that it lacks the signals required for virus incorporation. Subsequent studies with virus particles purified from HFF cells confirmed the association of polypeptides from ORFs 4 and 63 with virus particles (data not shown). In Fig. 3b, the polypeptides corresponding to the products of ORF 4 (52 kDa) and ORF 63 (47 kDa) are indicated.

To support these results, virus particles obtained from Ficoll and potassium tartrate gradients were subjected to a third fractionation through 10 to 55% sucrose gradients, as previously described (24). Fractionation on sucrose gradients reflects a third physical gradient parameter for particle purification, as sucrose has osmotic properties considerably different from those of Ficoll. The entire gradient was fractionated, and the polypeptides present in each fraction were examined by SDS-PAGE and immunoblot analysis. Figure 5a shows the polypeptides detected in each fraction after SDS-PAGE and silver staining. The peak fractions of the gradient containing virus particles were fractions 10 and 11, as detected by virion polypeptides including the 155-kDa major capsid protein and the 175-kDa tegument protein. Immunoblot analyses of the whole gradient with antibodies to ORF 10 showed that the encoded structural 47-kDa polypeptide peaked in the same fraction as the virus particles did (Fig. 5b). No reactivities of a 130-kDa polypeptide were found with antibodies to ORF 29 (data not shown). Panels c and d of Fig. 5 show immunoblot analysis of the fractions spanning the virus particle peak with antibodies to ORFs 4 and 63, respectively. The polypeptides of each ORF were clearly present and peaked in the fractions that contained the virus particles (fractions 10 and 11). These data supported a tight association of the ORF 4 and ORF 63 polypeptides with virus particles.

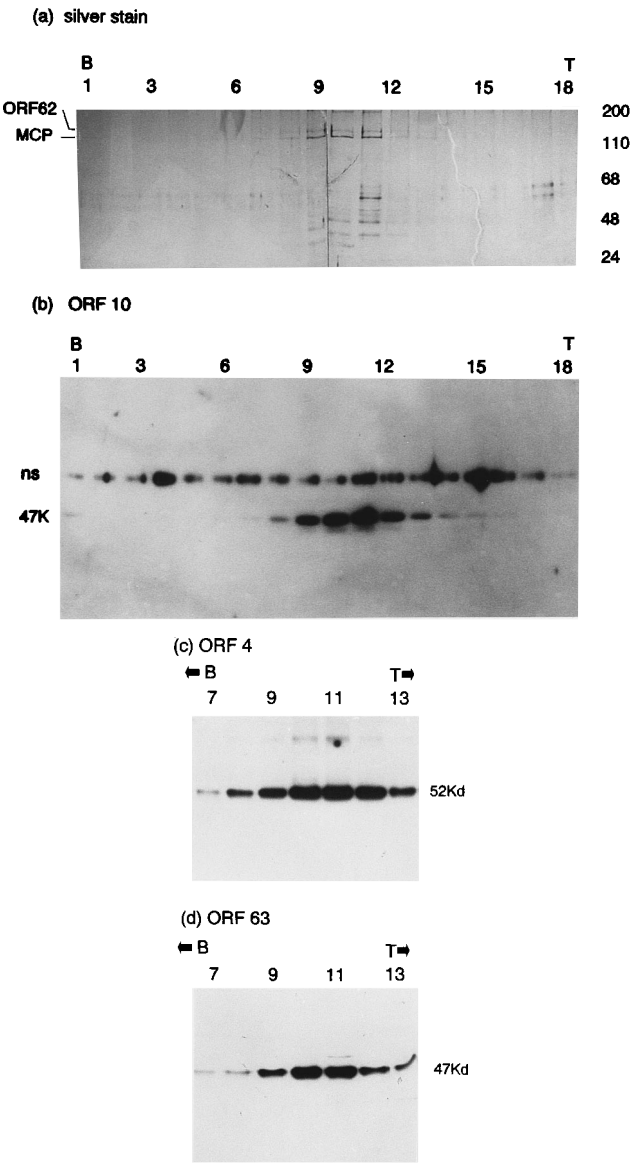

FIG. 5. Polypeptides of sucrose gradient-fractionated purified virus particles. (a) Silver-stained, SDS-PAGE-separated polypeptides present in fractions of the sucrose gradient, with fraction 1 representing the bottom of the gradient (B) and fraction 18 representing the top of the gradient (T). The positions of the major capsid protein (MCP) and the ORF 62 protein (ORF62) are indicated to the left, and the approximate positions of a set of molecular mass markers are indicated to the right in kilodaltons. (b) Immunoreactivity of an immunoblot of the same fractions after probing with antibodies to ORF 10. The 47-kDa ORF 10 polypeptide species and a nonspecifically reacting polypeptide (ns) are indicated. (c and d) Analyses of the peak fractions of the sucrose gradient shown in panel a for immunoreactivity with antibodies to ORF 4 (c) and ORF 63 (d), as detected by immunoblot analyses. The gradient fractions analyzed correlate with the fractions of the gradient shown in panels a and b. The size of the major polypeptide detected is indicated to the right of each blot in kilodaltons.

Viral location of the polypeptides of ORFs 4 and 63. The herpesvirus particle is composed of four structural units. A central DNA-protein core containing the DNA genome is surrounded by a 172-unit icosahedral shell which forms the nucleocapsid of the particle. Surrounding this is a region with a little-known defined structure called the tegument, which is further enclosed by a lipid envelope containing viral glycoproteins. The polypeptides of isolated VZV nucleocapsids were first examined for reactivity with antibodies to ORFs 4 and 63. Figure 3c shows [^{35}S]methionine-labelled polypeptides of highly purified VZV nucleocapsids, obtained by the method of Gibson and Roizman (17). Two gradients were required for the purification, as an initial single gradient revealed numerous cellular polypeptides in the preparation (Fig. 3c, G1). In the nucleocapsids obtained from the second gradient (G2), five polypeptides, of which the most abundant was the 155-kDa major capsid protein, were identified. Minor species of 32, 35, 54, and 57 kDa were also identified. On analysis by immunoblot, no reactivities of nucleocapsid polypeptides with antibodies to ORFs 4, 63, 10, and 62 were found (data not shown), suggesting that ORFs 4 and 63 were located in the viral envelope or the tegument.

To resolve this further, the distribution of the ORF 4 and ORF 63 polypeptides in the soluble and insoluble fractions of virus particles was examined after treatment with the detergents Nonidet P-40 and deoxycholate (Fig. 6). It was reasoned

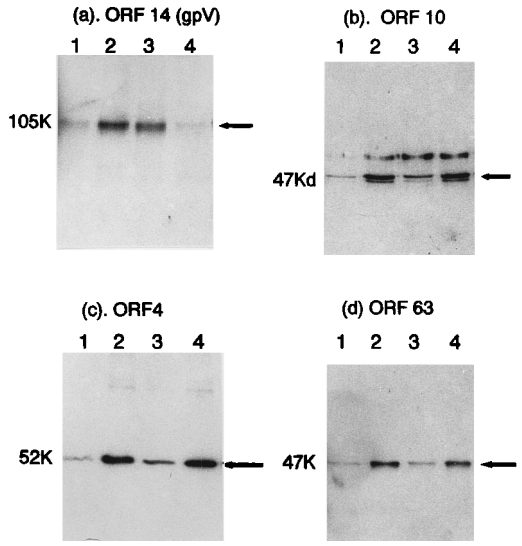

FIG. 6. Immunoreactivities of antibodies to ORF 14 (a), ORF 10 (b), ORF 4 (c), and ORF 63 (d) with identical immunoblots of electrophoretically separated polypeptides of VZV particles after no treatment (lanes 1 and 2) and treatment with 1% Nonidet P-40 and 0.5% deoxycholate (lanes 3 and 4). Equal amounts of the soluble fractions (lanes 1 and 3) and the pelleted insoluble material (lanes 2 and 4) were electrophoresed. The sizes of the major polypeptides detected are indicated in kilodaltons.

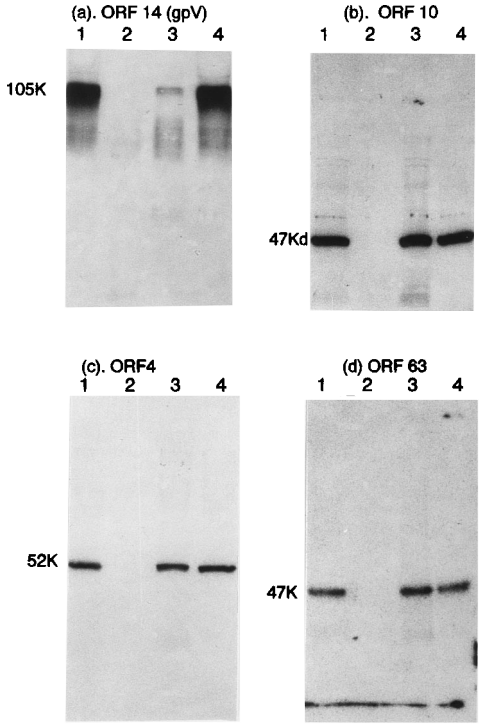

FIG. 7. Immunoreactivities of antibodies to ORF 14 (a), ORF 10 (b), ORF 4 (c), and ORF 63 (d) with identical immunoblots of electrophoretically separated polypeptides of VZV particles after no treatment (lanes 1) and treatment with trypsin in the presence of 1% Nonidet P-40 (lanes 2), with trypsin alone (lanes 3), and with 1% Nonidet P-40 alone (lanes 4). The sizes of the major polypeptide species detected are indicated in kilodaltons.

that if the ORF 4 and ORF 63 polypeptides were associated with the outside or inside of the envelope, solubilization by these detergents would likely release the proteins into the soluble fraction. Antibodies to the membrane glycoprotein gpV from ORF 14 and to the tegument protein from ORF 10 were used as controls. With antibodies to gpV, >90% of the 100,000- to 105,000-molecular-weight polypeptide was solubilized by treatment with detergents (Fig. 6a, lane 3); <10% was released without such treatment (lane 1) (as determined by estimation of the amount of ^{125}I-labelled protein A bound to the blot). The latter result may be due to a fraction of virus particles that failed to pellet in the centrifugation step. In contrast, the majority (>75%) of the tegument protein from ORF 10 remained in the insoluble fraction after detergent treatment and pelleted with the nucleocapsid-tegument (Fig. 6b, lane 4). A small fraction above levels obtained without treatment was released by the detergents (Fig. 6b, lane 3); this may reflect a partial disruption by the detergent treatment of the protein-protein interactions that maintain the tegument structure. Similar observations regarding the HSV-1 tegument protein αTIF have been reported (62). The 60-kDa polypeptide recognized by the ORF 10 antibodies was likely nonspecific. Probing of identical blots with antibodies to ORFs 4 and 63 revealed that the majority of the respective polypeptides failed to be solubilized by the detergents and remained predominantly in the insoluble fraction (Fig. 6c and d, respectively, lanes 4), strongly supporting a tegument location of these polypeptides. Harsher treatments of virus particles with higher concentrations of deoxycholate (up to 2%), 5 M NaCl, or 0.2% SDS also failed to completely solubilize the tegument proteins from ORFs 10, 4, and 63, suggesting more than just a loose association (data not shown). However, we were unable to resolve the detergent-treated virus particles by further gradients; we speculate that this might have resulted from some aggregation of virus particles following detergent treatment, causing them to sediment heterogeneously.

Further evidence suggesting a tegument location of the ORF 4 and ORF 63 polypeptides was obtained from trypsin protection studies. It was reasoned that tegument polypeptides would be protected from the protease by the virion envelope. However, such proteins would be rendered susceptible to digestion following disruption of the envelope by mild detergent treatment. For this analysis, virus particles were purified without protease inhibitors and were subjected to trypsin treatment in the presence or absence of 1% Nonidet P-40 as outlined in Materials and Methods. The results are presented in Fig. 7, in which four identical blots of the SDS-PAGE-separated polypeptides of the treated virus particle fractions probed with antibodies to ORFs 14 (gpV), 10, 4, and 63 are shown. Antibodies to ORF 14 (gpV) indicate that the majority of the glycoprotein was sensitive to trypsin digestion, both in the absence (Fig. 7a, lane 3) and in the presence (lane 2) of Nonidet P-40, as would be expected of a surface glycoprotein. A small fraction of the glycoprotein appears more resistant to digestion in the absence of detergents, which might reflect either a small amount of gpV on the inner surface of the viral envelope or, more likely, an increased susceptibility of the glycoprotein to digestion following detergent treatment. In contrast, the ORF 10 tegument protein was completely resistant to trypsin unless the envelope was solubilized (Fig. 7b). When identical blots were probed with antibodies to ORF 4 and ORF 63 (Fig. 7c and d, respectively), results similar to those for the tegument protein of ORF 10 were obtained, with the majority of the reactive polypeptides remaining completely resistant to trypsin digestion in the absence of detergents. These results strongly substantiate the conclusion that ORFs 4 and 63 encode polypeptides associated with the tegument of

VZV particles, with the viral envelope protecting the polypeptides from the external environment.

DISCUSSION

In this study, evidence was presented to strongly suggest that forms of polypeptides encoded by VZV ORFs 4 and 63, but not ORF 61, were tightly associated with virus particles, most likely as part of the tegument. While the function of these structural forms has not yet been resolved, we speculate that they might have roles, as transcriptional regulatory proteins, in the initial events of cell infection by VZV.

In the characterization of the antibodies required for these studies, an interesting cell-specific difference in the accumulation rates of the polypeptides from ORF 61 was identified. While the characterizations of polypeptides from ORFs 4 and 63 were straightforward and agreed with results of previous studies (42), the lack of detectable ORF 61 polypeptides in VZV-infected HFF cells was not expected. Three possible reasons could account for the results: the polypeptides were poorly expressed, were expressed but subject to a high rate of turnover, or were expressed but were not reactive with the antibodies. Low levels of ORF 61 polypeptides in VZV-infected cells have been noted by others (55). Initial studies of ORF 61 transcripts suggest that similar levels of ORF 61 RNAs accumulate in VZV-infected HFF and MeWo cells (57a). Furthermore, HFF cells infected with the v61 recombinant express 62- to 66-kDa polypeptides, suggesting that an antigenic difference is unlikely. We therefore suspect that ORF 61 proteins are translated inefficiently or are much less stable in this cell type than are those expressed in VZV-infected MeWo cells. While this issue is not yet resolved, these studies were important as they directed us to the use of VZV-infected MeWo cells for virus purification.

The conclusion that polypeptides encoded by ORFs 4 and 63, but not ORF 61, were associated with virus particles was based upon antibody reactivities with enveloped virus particles isolated from three successive gradient fractionations. We consider that such a strategy eliminated the possibility that contaminating membrane vesicles could account for these results, as each gradient has quite different parameters governing separation. We are, however, cautious in describing the virus particles, for two reasons. First, we were routinely unable to retain virus infectivity following the initial cytoplasmic extraction of the infected cells. The loss of infectivity is typical of VZV grown in culture (59) and has been suggested to occur as a consequence of viral particle maturation through the lysosomal vesicles, which, when released by cell lysis, degrade surface proteins on the virus which are required for infectivity (16). This also highlights our second reservation, in that these studies were performed with cell culture-grown VZV. VZV isolated from the vesicle fluid is considerably more infectious and stable than is culture-grown virus (59), and it is possible that maturation and egress occur differently for vesicle fluid-isolated virus than for virus grown in culture and that the virus may acquire different tegument proteins. Resolution of this problem could be difficult, because it is impractical to purify VZV from human vesicle fluid. However, immunoelectron microscopic techniques may allow the analysis of vesicle samples, and such approaches are currently being explored. Regarding the virus analyzed in this study, we further acknowledge the lack of quantitation of the levels of these proteins in the virus particle, due to the apparent migration of several polypeptides in the size range of the ORF 63 and ORF 4 polypeptides. In particular, the ORF 10 tegument polypeptide has mobility identical to that of the ORF 63 polypeptide (24). Assessment of the levels of these proteins might be achieved by using VZV mutants expressing size-altered polypeptides generated by insertions, but such viruses are not yet available.

Comparisons of the VZV structural-transcriptional regulatory proteins with homologs in related alphaherpesviruses suggest some similarities and some divergences. The VZV structural proteins from ORFs 10 and 62 have clearly diverged from their HSV-1 homologs. Unlike the essential HSV-1 αTIF (58), ORF 10 can be deleted without affecting virus growth (5), and while HSV-1 ICP4 is a minor virion component (60), the ORF 62 protein is abundant in virus (24). Regarding ORF 63, it has not yet been determined whether the HSV-1 homolog, ICP22, has forms which are virion associated. However, equine herpesvirus 1 particles have been shown to contain a form of the equine herpesvirus 1 protein considered homologous to HSV-1 ICP22 and VZV ORF 63 (18), suggesting some conservation of structural roles. HSV-1 particles contain low levels of ICP0 but contain no ICP27 (60, 61). The results for VZV suggest a divergence of ORFs 4 and 61 from the HSV-1 homologs, as ORF 61 (nonstructural) is homologous to HSV-1 ICP0 (structural) and the positional homolog of ORF 4 (structural) is HSV-1 ICP27 (nonstructural). However, the corresponding proteins are considerably divergent at both the amino acid and functional levels. Specifically, VZV ORF 4 cannot complement HSV-1 mutants altered in ICP27 and demonstrates considerable functional differences (8, 21, 22, 38, 44). Although ORF 61 can complement HSV-1 mutants with defective or absent ICP0 genes (36) and can activate transcription (39), it exhibits a powerful repressor activity on ORF 62- and ORF 4-mediated activation (41), an activity not found with HSV-1 ICP0. It therefore seems quite possible that the properties of the products of these genes have diverged regarding structural incorporation.

The question arises as to the roles, if any, that these proteins play in virus structure and/or regulation of IE gene expression. It is feasible that the proteins are nonspecifically acquired into the tegument by their localization to the site of tegument formation. However, we speculate that, as structural-transcriptional regulatory proteins, they may act during IE events of infection. It has been suggested that VZV IE genes might be activated in a manner different from that of HSV-1 IE genes, a possibility supported by the lack of a requirement for ORF 10 (5) and the finding of abundant levels of virion ORF 62 protein (24). Also indirectly supporting this is the observation that several proteins are made under IE conditions in VZV-infected cells (53). However, only the ORF 62 gene (of those genes homologous to HSV-1 IE genes) has TAATGARAT motifs in its promoter, the element responsible for IE gene expression in HSV-1 (28, 33, 34, 40). The fact that ORF 4 encodes a potent activator which can act in conjunction with the ORF 62 protein (8, 21, 22, 38, 44) is consistent with a model in which ORF 4 and ORF 62 proteins act in concert to stimulate transcription upon infection. A lack of ORF 61 polypeptides, as repressors of ORF 4- and ORF 62-mediated activation (41), is also consistent with this model. The functions of ORF 63 are less well understood. ORF 63 appears to encode transcriptional repression activities of the ORF 62 promoter and can regulate the activities of VZV early genes (23). However, it is possible that ORF 63 protein in conjunction with other structural proteins may exhibit different transcriptional regulatory functions, or that its activity differs in different cell types. Studies of HSV-1 ICP22 suggest functions which are essential for growth in certain cell types (51). Currently, we are investigating the possible interplay of ORF 63 proteins with other structural-transcriptional regulatory proteins in transcription from putative VZV IE gene promoters.

ACKNOWLEDGMENTS

This work was supported by a Public Health Service grant (EY 09397) from the National Eye Institute (P.R.K) and, in part, by a CORE grant for Vision Research (EY 08098) and by The Research to Prevent Blindness, Inc.

REFERENCES

1. **Ace, C. I., T. A. McKee, J. M. Cameron, and C. M. Preston.** 1989. Construction and characterization of a herpes simplex virus type 1 mutant unable to transinduce immediate-early gene expression. J. Virol. **63:**2260–2269.
2. **Cabirac, G. F., R. Mahalingam, M. Wellish, and D. H. Gilden.** 1990. Transactivation of viral tk promoters by proteins encoded by varicella zoster virus open reading frames 61 and 62. Virus Res. **15:**57–68.
3. **Campbell, M. E. M., J. W. Palfreyman, and C. M. Preston.** 1984. Identification of herpes simplex virus DNA sequences which encode a trans-acting polypeptide responsible for stimulation of immediate-early transcription. J. Mol. Biol. **180:**1–19.
4. **Chakrabarti, S., K. Brechling, and B. Moss.** 1986. Vaccinia virus expression vector: coexpression of β-galactosidase provides visual screening of recombinant virus plaques. Mol. Cell. Biol. **5:**3403–3409.
5. **Cohen, J. I., and K. Seidel.** 1994. Varicella-zoster virus (VZV) open reading frame 10 protein, the homolog of the essential herpes simplex virus protein VP16, is dispensable for VZV replication in vitro. J. Virol. **68:**7850–7858.
6. **Davison, A. J.** 1991. Varicella zoster virus. J. Gen. Virol. **72:**475–486.
7. **Davison, A. J., and J. E. Scott.** 1986. The complete DNA sequence of varicella-zoster virus. J. Gen. Virol. **67:**1759–1816.
8. **Defechereux, P., L. Melen, L. Baudoux, M. P. Merville Louis, B. Rentier, and J. Piette.** 1993. Characterization of the regulatory functions of varicella-zoster virus open reading frame 4 gene product. J. Virol. **67:**4379–4385.
9. **Disney, G. H., and R. D. Everett.** 1990. A herpes simplex virus type 1 recombinant with both copies of the Vmw175 coding sequences replaced by the homologous varicella-zoster virus open reading frame. J. Gen. Virol. **71:**2681–2689.
10. **Disney, G. H., T. A. McKee, C. M. Preston, and R. D. Everett.** 1990. The product of varicella zoster virus gene 62 autoregulates its own promoter. J. Gen. Virol. **71:**2999–3003.
11. **Dumas, A., J. Green, W. Marris, and J. Van der Norden.** 1980. Infectivity and molecular weight of varicella zoster virus DNA. J. Gen. Virol. **47:**233–235.
12. **Felser, J. M., P. R. Kinchington, G. Inchauspe, S. E. Straus, and J. M. Ostrove.** 1988. Cell lines containing varicella-zoster virus open reading frame 62 and expressing the "IE" 175 protein complement ICP4 mutants of herpes simplex virus type 1. J. Virol. **62:**2076–2082.
13. **Felser, J. M., S. E. Straus, and J. M. Ostrove.** 1987. Varicella-zoster virus complements herpes simplex virus type 1 temperature-sensitive mutants. J. Virol. **61:**225–228.
14. **Forghani, B., R. Mallingham, A. Vaffai, J. W. Hurst, and K. W. Dupuis.** 1990. Monoclonal antibody to immediate early protein encoded by varicella zoster virus gene 62. Virus Res. **16:**195–210.
15. **Gerster, T., and R. G. Roeder.** 1988. A herpes virus transactivating protein interacts with transcription factor OTF-1 and other cellular proteins. Proc. Natl. Acad. Sci. USA **85:**6347–6351.
16. **Gershon, A. A., D. L. Sherman, Z. Zhu, C. A. Gabel, R. T. Abron, and M. D. Gershon.** 1994. Intracellular transport of newly synthesized varicella-zoster virus: final envelopment in the *trans*-Golgi network. J. Virol. **68:**6372–6390.
17. **Gibson, W., and B. Roizman.** 1972. Proteins specified by herpes simplex virus. VIII. Characterization and composition of multiple capsid forms of subtypes 1 and 2. J. Virol. **10:**1044–1054.
18. **Holden, V. R., G. B. Caughman, Y. Zhao, R. N. Harty, and D. J. O'Callaghan.** 1994. Identification and characterization of the ICP22 protein of equine herpesvirus 1. J. Virol. **68:**4329–4340.
19. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14:**8–19.
20. **Honess, R. W., and B. Roizman.** 1975. Regulation of herpesvirus macromolecular synthesis. II. Sequential transition of polypeptide synthesis requires functional polypeptides. Proc. Natl. Acad. Sci. USA **72:**1276–1280.
21. **Inchauspe, G., S. Nagpal, and J. M. Ostrove.** 1989. Mapping of two varicella-zoster virus-encoded genes that activate the expression of viral early and late genes. Virology **173:**700–709.
22. **Inchauspe, G., and J. M. Ostrove.** 1989. Differential regulation by varicella-zoster virus and herpes simplex virus type-1 trans-activating genes. Virology **173:**710–714.
23. **Jackers, P., P. Defechereux, L. Baudoux, C. Lambert, M. Masser, M. P. Merville-Louis, B. Rentier, and J. Piette.** 1992. Characterization of regulatory functions of the varicella-zoster virus gene 63-encoded protein. J. Virol. **66:**3899–3903.
24. **Kinchington, P. R., J. K. Houghland, A. M. Arvin, W. T. Ruyechan, and J. Hay.** 1992. The varicella-zoster virus immediate-early protein IE62 is a major component of virus particles. J. Virol. **66:**359–366.
25. **Kinchington, P. R., G. Inchauspe, J. H. Subak Sharpe, F. Robey, J. Hay, and W. T. Ruyechan.** 1988. Identification and characterization of a varicella-zoster virus DNA-binding protein by using antisera directed against a predicted synthetic oligopeptide. J. Virol. **62:**802–809.
26. **Kinchington, P. R., P. Ling, M. Pensiero, B. Moss, W. T. Ruyechan, and J. Hay.** 1990. The glycoprotein products of VZV gene 14 and their defective accumulation in a vaccine strain (Oka). J. Virol. **64:**4540–4548.
27. **Kinchington, P. R., J. Remenick, J. M. Ostrove, S. E. Straus, W. T. Ruyechan, and J. Hay.** 1986. Glycoprotein gene of varicella-zoster virus with variable copy numbers of a 42-base-pair repeat sequence has homology to herpes simplex virus glycoprotein. C. J. Virol. **59:**660–668.
28. **Kinchington, P. R., J.-P. Vergnes, P. Defechereux, J. Piette, and S. E. Turse.** 1994. Transcriptional mapping of the varicella-zoster virus regulatory genes encoding open reading frames 4 and 63. J. Virol. **68:**3570–3581.
29. **Kristie, T., and B. Roizman.** 1987. Host cell proteins bind to the *cis* acting site required for virion-mediated induction of herpes simplex virus α genes. Proc. Natl. Acad. Sci. USA **84:**71–75.
30. **Lopetegui, P., H. Campo-Vera, and K. Yamanishi.** 1985. Varicella zoster virus specific polypeptides detected in cells treated with metabolic inhibitors. Microbiol. Immunol. **29:**569–575.
31. **Mackem, S., and B. Roizman.** 1982. Structural features of the herpes simplex virus α gene 4, 0, and 27 promoter-regulatory sequences which confer α regulation on chimeric thymidine kinase genes. J. Virol. **44:**939–949.
32. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. Complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. **69:**1531–1574.
33. **McKee, T. A., G. H. Disney, R. D. Everett, and C. M. Preston.** 1990. Control of expression of the varicella-zoster virus major immediate early gene. J. Gen. Virol. **71:**897–906.
34. **McKee, T. A., and C. M. Preston.** 1991. Identification of two protein binding sites within the varicella-zoster virus major immediate early gene promoter. Virus Res. **20:**59–69.
35. **McKnight, J. L. C., T. M. Kristie, and B. Roizman.** 1987. Binding of the virion protein mediating α gene induction in herpes simplex virus 1 infected cells to its *cis* site requires cellular proteins. Proc. Natl. Acad. Sci. USA **84:**7061–7065.
36. **Moriuchi, H., M. Moriuchi, H. A. Smith, S. E. Straus, and J. I. Cohen.** 1992. Varicella-zoster virus open reading frame 61 protein is functionally homologous to herpes simplex virus type 1 ICP0. J. Virol. **66:**7303–7308.
37. **Moriuchi, H., M. Moriuchi, S. E. Straus, and J. I. Cohen.** 1993. Varicella-zoster virus open reading frame 10 protein, the herpes simplex virus VP16 homolog, transactivates herpesvirus immediate-early gene promoters. J. Virol. **67:**2739–2746.
38. **Moriuchi, H., M. Moriuchi, H. A. Smith, and J. I. Cohen.** 1994. Varicella-zoster virus open reading frame 4 is functionally distinct from and does not complement its herpes simplex virus homolog ICP27. J. Virol. **68:**1987–1992.
39. **Moriuchi, H., M. Moriuchi, S. E. Straus, and J. I. Cohen.** 1993. Varicella-zoster virus (VZV) open reading frame 61 protein transactivates VZV gene promoters and enhances the infectivity of viral DNA. J. Virol. **67:**4290–4295.
40. **Moriuchi, M., H. Moriuchi, S. E. Straus, and J. I. Cohen.** 1994. Varicella zoster virus virion associated transactivator open reading frame 62 protein enhances the infectivity of VZV DNA. Virology **200:**297–300.
41. **Nagpal, S., and J. M. Ostrove.** 1991. Characterization of a potent varicella-zoster virus-encoded *trans*-repressor. J. Virol. **65:**5289–5296.
42. **Ng, T. I., L. Keenan, P. R. Kinchington, and C. Grose.** 1994. Phosphorylation of varicella-zoster virus open reading frame (ORF) 62 regulatory product by viral ORF 47-associated protein kinase. J. Virol. **68:**1350–1359.
43. **O'Hare, P. C., C. R. Goding, and A. Haigh.** 1988. Direct combinatorial interaction between a herpes simplex virus regulatory protein and a cellular octamer binding factor mediates specific induction of virus immediate early gene expression. EMBO J. **7:**4231–4238.
44. **Perera, L. P., S. Kaushal, P. R. Kinchington, J. D. Mosca, G. S. Hayward, and S. E. Straus.** 1994. Varicella-zoster virus open reading frame 4 encodes a viral transcriptional activator that is functionally distinct from that of herpes simplex virus homolog ICP27. J. Virol. **68:**2468–2477.
45. **Perera, L. P., J. D. Mosca, W. T. Ruyechan, and J. Hay.** 1992. Regulation of varicella-zoster virus gene expression in human T lymphocytes. J. Virol. **66:**5298–5304.
46. **Perera, L. P., J. D. Mosca, M. Sadeghi-Zadeh, W. T. Ruyechan, and J. Hay.** 1992. The varicella-zoster virus immediate-early protein IE62 can positively regulate its cognate promoter. Virology **191:**346–354.
47. **Post, L. E., S. Mackem, and B. Roizman.** 1981. Regulation of α genes of herpes simplex virus; expression of chimeric genes produced by fusion of thymidine kinase with α gene promoters. Cell **24:**555–565.
48. **Preston, C. M., M. C. Frame, and M. E. M. Campbell.** 1988. A complex formed between cell components and an HSV structural polypeptide binds to a viral immediate early gene regulatory DNA sequence. Cell **52:**425–434.
49. **Roizman, B., and D. Spector.** 1992. The induction of α genes by the α trans-inducing factor, p. 17–28. *In* E. K. Wagner (ed.), Herpesvirus transcription and its regulation. CRC Press, Boca Raton, Fla.
50. **Ruyechan, W. T., P. Ling, P. R. Kinchington, and J. Hay.** 1992. The correlation between varicella zoster virus transcription and the sequence of the viral genome, p. 301–317. *In* E. K. Wagner (ed.), Herpesvirus transcription

and its regulation. CRC Press, Boca Raton, Fla.
51. **Sears, A. E., I. W. Halliburton, B. Meignier, S. Silver, and B. Roizman.** 1985. Herpes simplex virus mutant deleted in α22 gene; growth and gene expression in permissive and restricted cells and establishment of latency in mice. J. Virol. **59:**7310–7314.
52. **Shemer, Y., S. Levinson-Kriss, and I. Sarov.** 1980. Isolation and polypeptide characterization of varicella zoster virus. Virology **106:**133–140.
53. **Shiraki, K., and R. W. Hyman.** 1987. The immediate early proteins of varicella zoster virus. Virology **156:**423–426.
54. **Shiraki, K., and M. Takahashi.** 1982. Virus particles and glycoproteins excreted from cultured cells infected with varicella zoster virus (VZV). J. Gen. Virol. **61:**271–275.
55. **Stevenson, D., K. L. Colman, and A. J. Davison.** 1992. Characterization of the varicella-zoster virus gene 61 protein. J. Gen. Virol. **73:**521–530.
56. **Straus, S. E., H. S. Aulalkh, W. T. Ruyechan, J. Hay, T. A. Casey, G. F. VanDe Woude, J. Owens, and H. A. Smith.** 1981. Structure of varicella-zoster virus DNA. J. Virol. **40:**516–525.
57. **Szilagyi, J. F., and C. Cunningham.** 1991. Identification and characterization of a novel non-infectious herpes simplex virus related particle. J. Gen. Virol. **72:**661–668.
57a. **Vergnes, J. P., and P. R. Kinchington.** Unpublished data.
58. **Weinheimer, S. P., B. A. Boyd, S. K. Durham, J. L. Resnick, and D. R. O'Boyle.** 1992. Deletion of the VP16 open reading frame of herpes simplex virus type 1. J. Virol. **66:**258–269.
59. **Weller, T. H., H. M. Wilton, and E. J. Bell.** 1958. The etiological agents of varicella and herpes zoster. Isolation, propagation and cultural characteristics in vitro. J. Exp. Med. **108:**843–868.
60. **Yao, F., and R. J. Courtney.** 1989. A major transcriptional regulatory protein (ICP4) of herpes simplex virus type 1 is associated with purified virions. J. Virol. **63:**3338–3344.
61. **Yao, F., and R. J. Courtney.** 1992. Association of ICP0 but not ICP27 with purified virions of herpes simplex virus type 1. J. Virol. **66:**2709–2716.
62. **Zhang, Y., and J. L. C. McKnight.** 1993. Herpes simplex virus type 1 UL46 and UL47 deletion mutants lack VP11 and VP12 or VP13 and VP14, respectively, and exhibit altered viral thymidine kinase expression. J. Virol. **67:**1482–1492.
63. **Zhang, Y., D. A. Sirko, and J. L. C. McKnight.** 1991. Role of herpes simplex virus type 1 UL46 and UL47 in αTIF-mediated transcriptional induction: characterization of three viral deletion mutants. J. Virol. **65:**829–841.