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SHORT COMMUNICATION

The Acidic Amino-Terminal Region of Varicella-Zoster Virus Open Reading Frame 4 Protein Is Required for Transactivation and Can Functionally Replace the Corresponding Region of Herpes Simplex Virus ICP27

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Both varicella-zoster virus open reading frame 4 (ORF4) protein and its herpes simplex virus type 1 homolog ICP27 have highly acidic amino-terminal regions and cysteine-rich carboxy-terminal regions. To investigate the functional domains of these proteins, mutants were constructed and their transregulatory functions were tested in transient expression assays using two reporter plasmids, pTK-CAT-SV40A and pTK-CAT-synA, containing the same promoter sequences but different mRNA processing signals. ORF4 transactivates both pTK-CAT-SV40A and pTK-CAT-synA, while ICP27 transrepresses pTK-CAT-SV40A and transactivates pTK-CAT-synA. Deletion of the ORF4 amino-terminal region abolished most of the transactivating activity for pTK-CAT-synA but retained most of the transactivating activity for pTK-CAT-SV40A. Construction of chimeric ORF4–ICP27 molecules indicated that the ORF4 amino-terminal region was able to replace the corresponding region of ICP27 which is required for both transrepression of pTK-CAT-SV40A and transactivation of pTK-CAT-synA. Similarly, the ICP27 amino-terminal region was able to partially replace the corresponding region of ORF4 which is required for transactivation of pTK-CAT-synA. Thus, while ORF4 and ICP27 have different properties in transient expression assays, the amino-terminal regions of ORF4 and ICP27 are functionally homologous to each other and are important in regulating gene expression.

Varicella-zoster virus (VZV), a member of the human alphaherpesvirus family, is the etiologic agent of chickenpox and shingles. Complete DNA sequences of VZV and herpes simplex virus type 1 (HSV-1), another member of the human alphaherpesvirus family, indicate that many VZV gene products have HSV-1 counterparts, on the basis of gene location and predicted amino acid sequence (*1, 2*), allowing the functions of several VZV gene products to be deduced.

Previous studies have shown that VZV homologs of HSV-1 proteins involved in transcriptional regulation also play important roles in viral gene expression. VZV open reading frame 10 (ORF10) protein, the homolog of HSV-1 VP16 [which stimulates expression of HSV-1 immediate-early (IE) genes *in trans* (*3*)], is a component of the virus particle (*4*) and transactivates herpesvirus IE promoters in transient expression assays (*5*). VZV ORF62 protein is the homolog of HSV-1 IE protein ICP4 (*6, 7*) and transactivates a wide variety of promoters from all three putative kinetic classes (*8–10*) and enhances the infectivity of transfected VZV DNA (*11*). VZV ORF61 is functionally homologous to HSV-1 IE protein ICP0 (*12, 13, 13a*), despite the fact that amino acid sequence homology of these two proteins is limited to the RING finger domain located in their amino-termini (*1, 14*).

VZV ORF4 protein shares considerable amino acid sequence homology with HSV-1 IE protein ICP27, especially in the carboxy-terminus. The carboxy-terminal region of ICP27 is rich in cysteine and histidine residues and has been shown to bind zinc (*15*). While the carboxy region of ORF4 also contains cysteine and histidine residues, it is not known whether this region also binds zinc. The amino-terminal regions of these two proteins have limited amino acid homology; however, both are highly acidic (Figs. 1A and 1C).

Despite structural similarities, VZV ORF4 and HSV-1 ICP27 act differently in transient expression assays. ORF4 protein transactivates a wide variety of target contructs (*16–18*). In contrast, ICP27 acts as a transrepressor or a transactivator (*19, 20*), depending on the presence of different mRNA processing signals (*21*). Although ICP27 is required for HSV-1 replication (*22*), the role of ORF4 in viral infection is not well understood. Cell lines expressing ORF4 are unable to efficiently complement HSV-1 ICP27 mutants (*17*).

Several laboratories have shown that the carboxy-terminal region of ICP27 is required for the transregulatory

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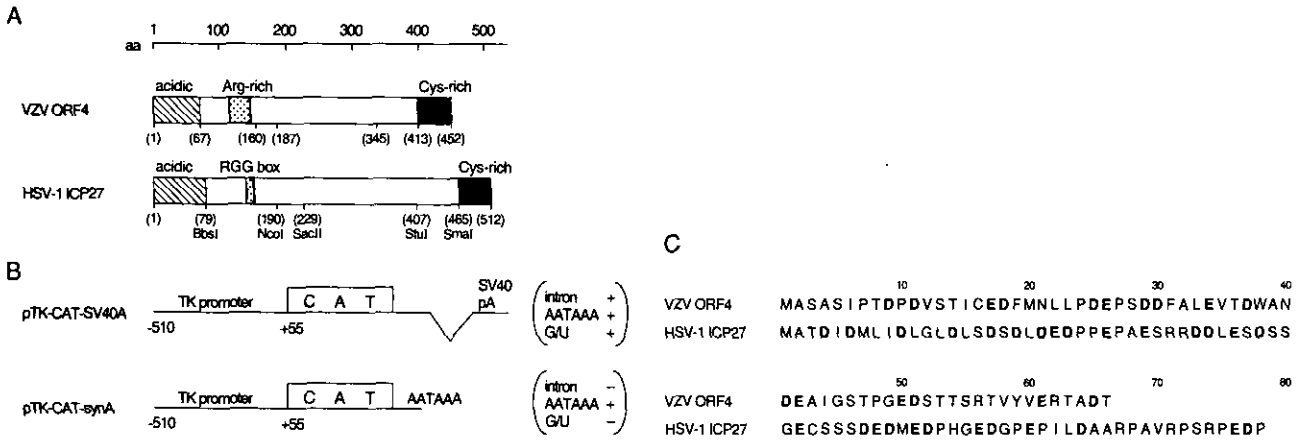

FIG. 1. (A) Structures of VZV ORF4 and HSV-1 ICP27. Both proteins contain highly acidic amino-terminal regions (hatched areas) and cysteine-rich carboxy-terminal regions (solid areas). ICP27 contains an arginine- and glycine-rich region (RGG box; stippled area), while the corresponding region of ORF4 is rich in arginine but not glycine. Numbers in parentheses are amino acid positions used for constructing mutants and chimeric proteins. ORF4 fragments were obtained using polymerase chain reaction, while ICP27 fragments were obtained using restriction digestion with the indicated enzymes. Coding sequences of all full-length, mutant, and chimeric ORF4 and ICP27 proteins were inserted into plasmid pCMV (*10*). (B) Structures of reporter plasmids. pTK-CAT-SV40A contains the HSV-1 thymidine kinase (TK) gene promoter sequence (−510 to +55 relative to the transcription start site) followed by the chloramphenicol acetyltransferase (CAT) gene, the simian virus 40 (SV40) small-t-antigen intron, and the SV40 early polyadenylation region (containing the conserved polyadenylation recognition signal AATAAA and the G/U box) (*21*). pTK-CAT-synA contains the HSV-1 TK promoter sequence followed by the CAT gene and a synthetic oligonucleotide (containing the conserved polyadenylation recognition signal AATAAA but no G/U box) (*21*). (C) Amino acid sequences of the amino-terminal regions of VZV ORF4 protein (*1*) and HSV-1 ICP27 (*2*). The predicted amino acid sequences are shown in single-letter code. The bold letters, D and E, indicate acidic residues.

functions of ICP27 (*23–25*). Recently, Rice *et al.* (*26*) showed that the amino-terminal region of ICP27 is also required for transrepressing activity of ICP27. Furthermore, both of these regions are critical for replication of HSV-1 (*24, 26, 27*). In contrast, functional domains of ORF4 have not been determined.

To further investigate the functional similarities and differences between VZV ORF4 and HSV-1 ICP27, mutant and chimeric proteins were expressed, and their transregulatory activity was determined using two target constructs, pTK-CAT-SV40A and pTK-CAT-synA, bearing the same [HSV-1 thymidine kinase (TK) gene] promoter sequence but distinct mRNA processing signals (Fig. 1B).

To localize functional domains of VZV ORF4, we constructed a series of amino- or carboxy-terminal truncation mutants of ORF4, and the transregulatory activity of these constructs was investigated by assessing their ability to transactivate pTK-CAT-SV40A and pTK-CAT-synA. Full-length ORF4 transactivated both reporter genes in the presence or absence of HSV-1 ICP0 and ICP4 (Fig. 2; data not shown). Deletion of the acidic region (amino acids 2 to 66) did not result in loss of transactivation of pTK-CAT-SV40A but markedly reduced the transactivating activity for pTK-CAT-synA. Deletion of amino acids 2 to 159 reduced the transactivating activity for pTK-CAT-SV40A by 60%, and further deletion of the amino-terminal region (amino acids 2 to 186 or further) abolished the transactivating activity for pTK-CAT-SV40A. Likewise, deletion of the cysteine-rich carboxy-terminal region (amino acids 404 to 452) resulted in loss of transactivating activity for both reporter genes (Fig. 2). Thus, while the cysteine-rich carboxy-terminal region is essential for the transactivating activity of full-length ORF4 protein, the acidic amino-terminal region is required for transactivation of pTK-CAT-synA but is dispensable for transactivation of pTK-CAT-SV40A.

Transregulatory function of these constructs was also tested with another set of reporter plasmids, p4CAT-SV40A and p4CAT-NP. Both plasmids contain the VZV ORF4 promoter sequence; however, p4CAT-SV40A, like pTK-CAT-SV40A, contains an intron, a G/U box, and the conserved polyadenylation signal AATAAA from the SV40 early polyadenylation region (*10*), while p4CAT-NP does not contain any eukaryotic mRNA-processing signal (*9*). Full-length ORF4 transactivated p4CAT-SV40A and p4CAT-NP up to 16- and 9-fold, respectively. Deletion of the acidic amino-terminal region (amino acids 2 to 66) retained the transactivating activity of p4CAT-SV40A up to 10-fold but abolished the transactivating activity of p4CAT-NP. Deletion of the carboxy-terminal region (amino acids 404 to 452) abolished the transactivating activity of both reporter genes (data not shown). These results confirm that the acidic amino-terminal region of ORF4 is essential for transactivation of reporter genes with absent (or minimal) polyadenylation signals but is not required for transactivation of reporter genes with polyadenylation signals.

To further investigate the function of the acidic amino-terminal regions of both proteins, ORF4–ICP27 chimeric proteins were constructed and their transregulatory function was evaluated in transient expression assays. While deletion of the acidic region (amino acids 2 to 66) of

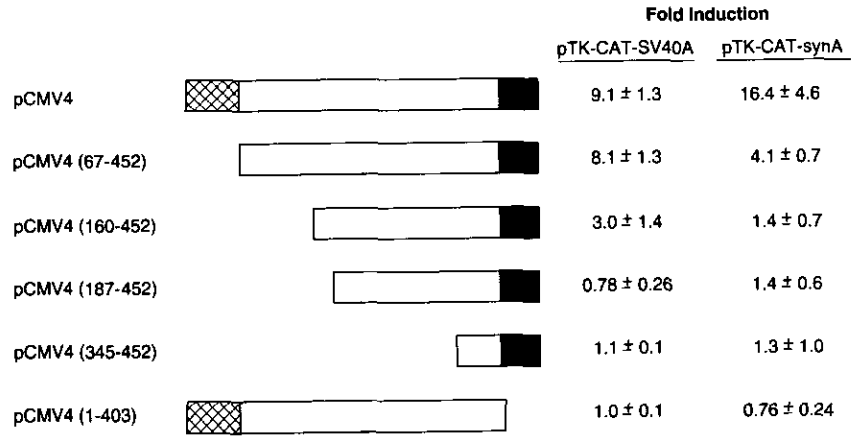

FIG. 2. Transregulatory function of amino- or carboxy-terminal truncation mutants of VZV ORF4. Vero cells were cotransfected with 2 μg of pTK-CAT-SV40A, 0.5 μg each of pK1-2 (ICP4) and pMC151 (ICP0) (*28*), and 1 μg of the indicated plasmid (column 1); or 5 μg of pTK-CAT-synA, 2.5 μg each of pK1-2 and pMC151, and 5 μg of the indicated plasmid (column 2). Transfection and CAT assays were performed as described previously (*12*). *Full-length ORF4 transactivated both pTK-CAT-SV40A and pTK-CAT-synA. Deletion of amino acids 2 to 66 of ORF4 abolished most of transactivat*ing activity for pTK-CAT-synA, but most of transactivating activity for pTK-CAT-SV40A was retained. More extensive amino-terminal deletions or a carboxy-terminal deletion of ORF4 resulted in loss of transactivation of both reporter plasmids. Boxes represent the protein coding sequences, cross-hatched boxes represent the acidic amino-terminal region, solid boxes represent the cysteine-rich carboxy-terminal region, and numbers in parentheses indicate amino acids. Fold induction is the CAT activity relative to that obtained for transfection with pCMV (vector control), pK1-2, pMC151, and pTK-CAT-SV40A or pTK-CAT-synA. The averages and the standard deviations of at least three experiments are shown.

ORF4 significantly reduced the transactivating activity for pTK-CAT-synA, fusion of the acidic region (amino acids 1 to 78) of ICP27 to the carboxy portion (amino acids 67 to 452) of ORF4 partially restored the transactivating activity for pTK-CAT-synA (pCMV27Ac/4; Fig. 3).

Full-length ICP27 transrepressed pTK-CAT-SV40A and transactivated pTK-CAT-synA, and deletion of the acidic region (amino acids 2 to 78) abolished both transrepressing and transactivating activity. Fusion of the acidic region (amino acids 1 to 66) of ORF4 to the carboxy portion (amino acids 79 to 512) of ICP27 restored the transrepressing and transactivating activity (pCMV4Ac/27; Fig. 3). Thus, the acidic amino-terminal domain of one protein can, at least in part, replace the corresponding domain of the other in transient expression assays.

To compare the functional similarities and differences of carboxy-terminal regions of these two proteins, two additional chimeric ORF4–ICP27 proteins were constructed and tested in transient expression assays. While deletion of the cysteine-rich region (amino acids 404 to 452) of ORF4 abolished the transactivating activity for both reporter genes, fusion of the corresponding region (amino acids 465 to 512) of ICP27 to the amino portion (amino acids 1 to 403) of ORF4 did not restore transactivating activity (pCMV4/27Cys; Fig. 4).

Deletion of the cysteine-rich region (amino acids 465

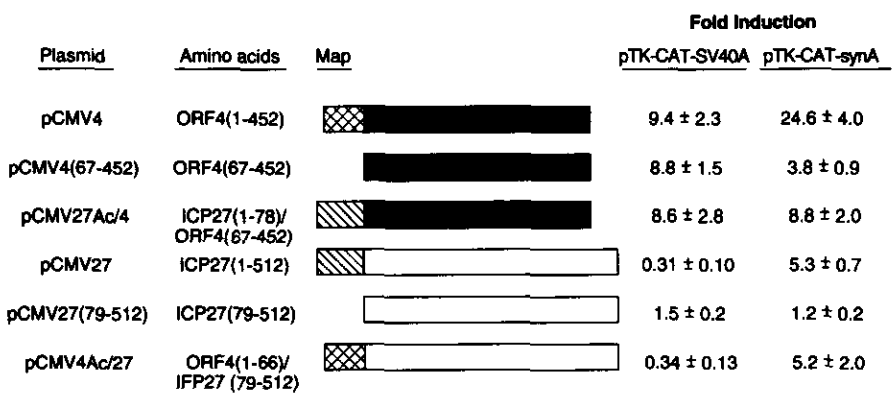

FIG. 3. Transregulatory function of chimeric ORF4–ICP27 proteins (substitution of acidic amino-terminal domains). Vero cells were transfected as described in legend of Fig. 2. The full-length ORF4 protein and the ICP27 [acidic (Ac) amino acids 1 to 78]–ORF4 (amino acids 67 to 452) fusion protein (pCMV27Ac/4) transactivated both pTK-CAT-SV40A and pTK-CAT-synA. The full-length ICP27 and the ORF4 (acidic amino acids 1 to 66)–ICP27 (amino acids 79 to 512) fusion protein (pCMV4Ac/27) transrepressed pTK-CAT-SV40A and transactivated pTK-CAT-synA. Cross-hatched boxes *represent the ORF4 acidic amino-terminal domain (amino acids 1 to 66), solid boxes represent the carboxy portion of ORF4 (amino acids 67 to* 452), hatched boxes represent the ICP27 acidic amino-terminal domain (amino acids 1 to 78), and open boxes represent the carboxy portion of ICP27 (amino acids 79 to 512). The averages and the standard deviations from at least three experiments are shown.

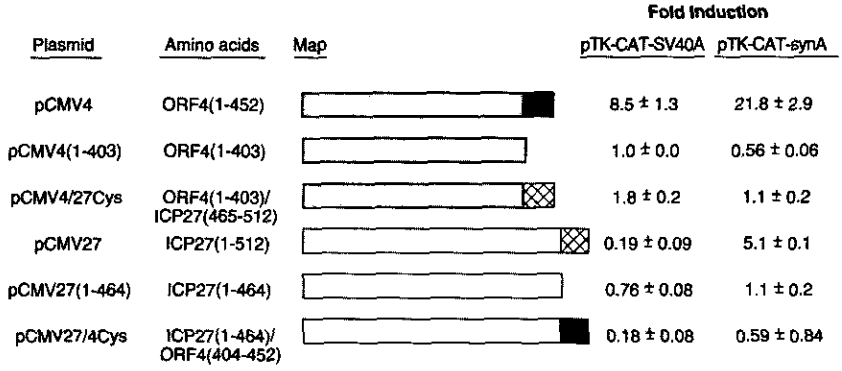

FIG. 4. Transregulatory function of chimeric ORF4–ICP27 proteins (substitution of cysteine-rich carboxy-terminal domains). Vero cells were transfected as described in legend of Fig. 2. The ORF4 (amino acids 1 to 403)–ICP27 [cysteine-rich (Cys) amino acids 465 to 512] fusion protein (pCMV4/27Cys) failed to transactivate either of the two reporter plasmids. The ICP27 (amino acids 1 to 464)–ORF4 (cysteine-rich amino acids 404 to 452) fusion protein (pCMV27/4Cys) transrepressed pTK-CAT-SV40A but failed to transactivate pTK-CAT-synA. Stippled boxes represent the amino portion (amino acids 1 to 403) of ORF4, solid boxes represent the ORF4 cysteine-rich carboxy-terminal domain (amino acids 404 to 452), open boxes represent the amino portion (amino acids 1 to 464) of ICP27, and cross-hatched boxes represent the ICP27 cysteine-rich carboxy-terminal domain (amino acids 465 to 512). The averages and the standard deviations from at least three experiments are shown.

to 512) of ICP27 resulted in loss of both transrepressing and transactivating activity of full-length ICP27. Fusion of the corresponding region of ORF4 (amino acids 404 to 452) to the amino portion (amino acids 1 to 464) of ICP27 restored the transrepressing activity, but not the transactivating activity of ICP27 (pCMV27/4Cys; Fig. 4). Thus, the cysteine-rich carboxy-terminal domains of these two proteins may have distinct properties.

Previous studies showed that both acidic amino-terminal and cysteine-rich carboxy-terminal domains of ICP27 are required for full viral replication (*24, 26, 27*). Therefore, we determined whether the amino or carboxy portion of ORF4 could substitute for the corresponding domain of ICP27 by assaying the ability of transfected chimeric ORF4–ICP27 genes to complement replication of an ICP27 deletion mutant.

Transfection of the full-length ICP27 gene complemented an HSV-1 ICP27 deletion mutant 5*d*/1.2 (*29*); however, expression of ORF4, an ICP27 amino-terminal truncation mutant, or an ICP27 carboxy-terminal truncation mutant could not efficiently complement the mutant (Table 1). Transfection of a chimeric plasmid (pCMV4Ac/27) expressing the acidic region (amino acids 1 to 66) of ORF4 fused to the carboxy portion (amino acids 79 to 512) of ICP27 resulted in a complementation index of 8.3 for the ICP27 mutant virus. This represents a 35-fold increase when compared to the complementation index (0.24) for the same construct lacking the acidic region of ORF4 [plasmid pCMV27(79–512)]. In contrast, transfection of a chimeric plasmid (pCMV27/4Cys) expressing amino acids 1 to 464 of ICP27 fused to the cysteine-rich region (amino acids 404 to 452) of ORF4 resulted in a complementation index of only 0.67. Thus, the acidic amino-terminal domain, but not the cysteine-rich carboxy-terminal domain of VZV ORF4, had modest activity when compared to the corresponding domain of HSV-1 ICP27 in the context of HSV-1 infection.

To examine subcellular localization of full-length, mutant, and chimeric ORF4 and ICP27 proteins, Vero cells were transfected with plasmids and stained with antibodies to ORF4 or ICP27. Full-length, amino-terminal-truncated (amino acids 79 to 512) and carboxy-terminal-truncated (amino acids 1 to 464) ICP27 each localized to the nucleus (*30*; Moriuchi and Moriuchi, unpublished data). In contrast, full-length and amino-terminal truncation mutants (amino acids 67 to 452) of ORF4 protein were found predominantly in the cytoplasm, but also, to a lesser extent, in the nucleus (Figs. 5A and 5B; data not shown). Also, in VZV-infected cells, ORF4 protein localized mainly to the cytoplasm (Defechereux *et al.*, unpublished data). Surprisingly, a carboxy-terminal truncation mutant (amino acids 1 to 403) of ORF4 was localized to the nucleus in

TABLE 1

Complementation of an HSV-1 ICP27 Deletion Mutant (5*d*/1.2) by Full-Length, Mutant, and Chimeric ICP27 and ORF4 Proteins

Plasmid transfected[a]	Virus yield (PFU/ml)[b]	Complementation index (%)[c]
pCMV	$<10^1$	<0.06
pCMV4	2.2×10^1	0.12
pCMV27	1.8×10^4	100
pCMV27 (79–512)	4.3×10^1	0.24
pCMV4Ac/27	1.5×10^3	8.3
pCMV27 (1–464)	$<10^1$	<0.06
pCMV27/4 Cys	1.2×10^2	0.67

[a] Approximately 5×10^5 Vero cells were transfected with 10 μg of the plasmid indicated. One day later, the cells were infected with 1 PFU of HSV-1 mutant 5*d*/1.2 per cell. The infected cells and medium were harvested 24 hr after infection and titrated.

[b] Virus titers were determined by plaque assay on Vero 3-3 cells (which express ICP27).

[c] Expressed as yield relative to that obtained for the transfection with pCMV27.

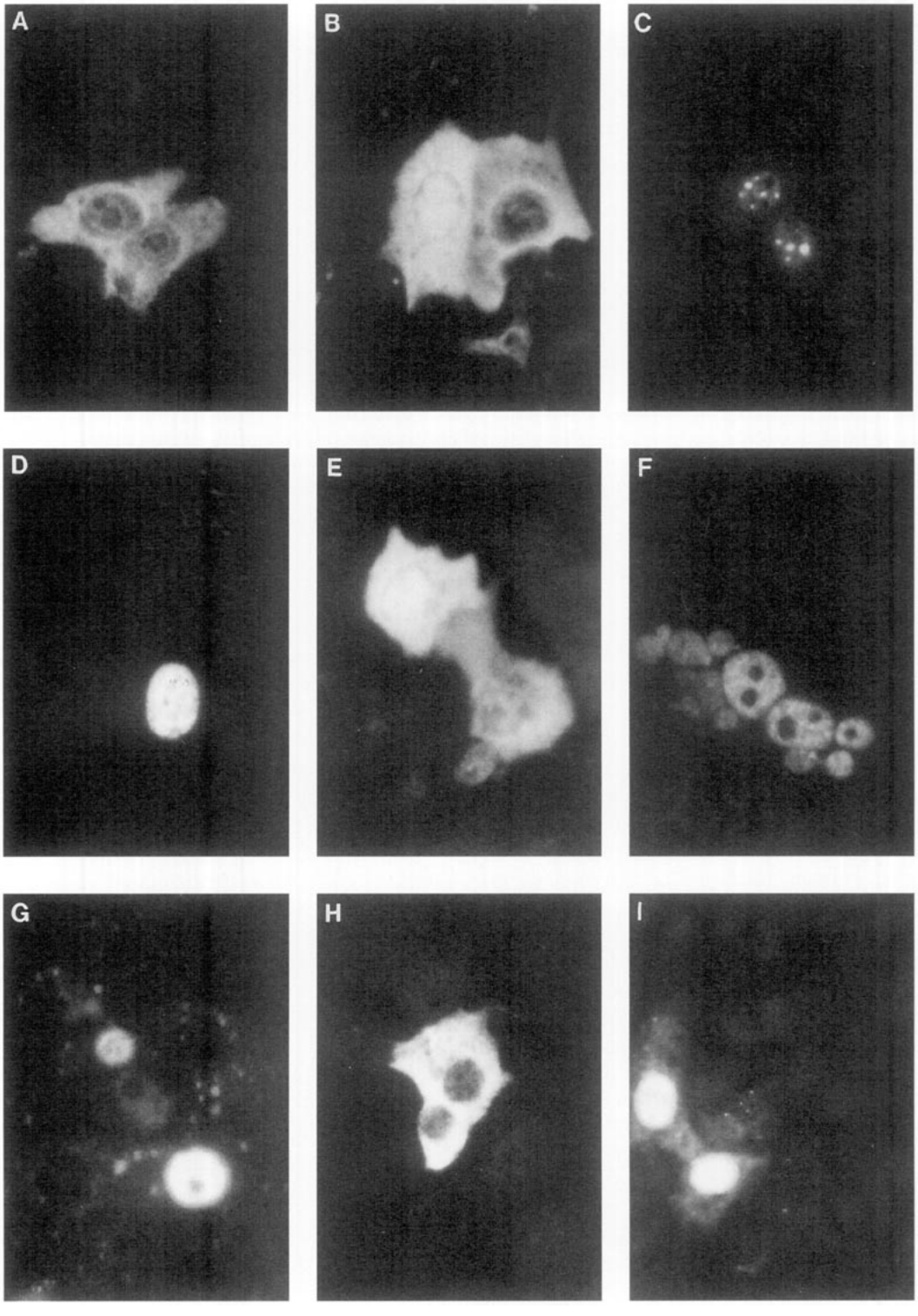

FIG. 5. Subcellular localization of full-length, mutant, and chimeric ORF4 and ICP27 proteins. Vero cells were transfected with plasmids expressing full-length ORF4 [pCMV4 (A)], an amino-terminal-truncation mutant of ORF4 [pCMV4(67–452) (B)], a carboxy-terminal-truncation mutant of ORF4 [pCMV4(1–403) (C)], the ORF4 (amino acids 1 to 66)–ICP27 (amino acids 79 to 512) fusion protein [pCMV4Ac/27 (D)], the ICP27 (amino acids 1 to 78)–ORF4 (amino acids 67 to 452) fusion protein [pCMV27Ac/4 (E)], the ORF4 (amino acids 1 to 403)–ICP27 (amino acids 465 to 512) fusion protein [pCMV4/27Cys (F)], the ICP27 (amino acids 1 to 464)–ORF4 (amino acids 404 to 452) fusion protein [pCMV27/4Cys (G)], the ORF4 [amino acids 1 to 159 (which contain the arginine-rich sequence at amino acids 115 to 143)]–ICP27 (amino acids 190 to 512) fusion protein [pCMV4RGG/27 (H)], and the ICP27 [amino acids 1 to 189 (which contains the arginine-glycine-rich sequence [RGG box] at amino acids 138 to 152)]–ORF4 (amino acids *160 to 452) fusion protein [pCMV27RGG/4 (I)]. Transfected cells were stained with rabbit antibody to ORF4 (amino acids 439 to 452)* (A, B, E, and I), or rabbit antibody to ORF4 (amino acids 169 to 181) (C and F), or mouse monoclonal anti-ICP27 antibody (D, G, and H). Subcellular localization of the proteins is summarized in Table 2.

TABLE 2

Subcellular Localization of Full-Length, Mutant, and Chimeric VZV ORF4 and HSV-1 ICP27

Plasmid	Amino acids[a]	Subcellular localization[b]
pCMV4	ORF4 (1–452)	C>N
pCMV4 (67–452)	ORF4 (67–452)	C>N
pCMV4 (1–403)	ORF4 (1–403)	N (nucleolar exclusion, speckled)
pCMV27	ICP27 (1–512)	N
pCMV27 (79–512)	ICP27 (79–512)	N
pCMV27 (1–464)	ICP27 (1–464)	N
pCMV4Ac/27	ORF4 (1–66)/ICP27 (79–512)	N
pCMV27Ac/4	ICP27 (1–78)/ORF4 (67–452)	C>N
pCMV4/27 Cys	ORF4 (1–403)/ICP27 (465–512)	N (nucleolar exclusion, diffuse)
pCMV27/4 Cys	ICP27 (1–464)/ORF4 (404–452)	N
pCMV4RGG/27	ORF4 (1–159)/ICP27 (190–512)	C
pCMV27RGG/4	ICP27 (1–189)/ORF4 (160–452)	N

[a] Numbers in parentheses indicate amino acids.
[b] C, cytoplasmic; N, nuclear; C>N, predominantly cytoplasmic.

a speckled pattern with nucleolar exclusion and was absent from the cytoplasm (Fig. 5C). These findings suggest that the amino portion (amino acids 1 to 403) of ORF4 protein contains a nuclear localization signal and that the carboxy portion (amino acids 404 to 452) may contribute to cytoplasmic localization.

Each of the chimeric ORF4–ICP27 mutants localized exclusively to the nucleus (Figs. 5D, 5F, 5G, and 5I) with two exceptions. The ORF4 (amino acids 1 to 159)–ICP27 (amino acids 190 to 512) fusion protein localized exclusively to the cytoplasm (Fig. 5H), while the ICP27 (amino acids 1 to 78)–ORF4 (amino acids 67 to 452) fusion protein localized to both the cytoplasm and the nucleus (Fig. 5E; Table 2).

The present study demonstrates that the acidic amino-terminal region of ORF4 is essential for transactivation of reporter genes carrying minimal polyadenylation signals, but that this region is not required for transactivation of reporter genes carrying more efficient polyadenylation signals. The corresponding amino-terminal region of HSV-1 ICP27 has previously been shown to be important for transregulatory functions in transient expression assays (*26*). While the amino acid sequences of the amino-terminal regions of ORF4 and ICP27 are not highly conserved, these regions can functionally substitute for each other in transient expression assays.

The amino-terminal region of VZV ORF4 is highly acidic. Sixteen of the first 66 amino acids in ORF4 are either aspartic or glutamic acid, and seven residues are serine which, if phosphorylated, may be negatively charged. Net acidity is characteristic of several transcriptional activation regions; however, other critical structural features are also required for transactivating activity (for review, see *31*). While the amino-terminal regions of the VZV ORF4, HSV-1 ICP27, equine herpesvirus 1 (EHV-1) *UL3* (*32*), and their gammaherpesvirus homologs (*33, 34*) are all acidic, they share little amino acid homology. The acidic regions of ORF4 (*18*) and ICP27 (Moriuchi and Moriuchi, unpublished data) did not act as efficient transcriptional activation domains when fused to the GAL4 DNA-binding region. Furthermore, linker-insertion mutageneis of the amino-terminus of ICP27 showed that in-frame insertions in the amino-terminus of ICP27 did not abolish transregulatory functions of the protein (*23*). Therefore, these studies suggest that the net charge of the amino-terminus may be more important than any conserved structural motif in the ability of these regions to contribute to transactivation of target genes.

In contrast to the amino-terminal region, the amino acid sequences of the cysteine-rich carboxy-terminal regions are highly conserved among ORF4 homologs. The carboxy-terminal region is essential for the transregulatory function of VZV ORF4 (*18*; this study) and HSV-1 ICP27 (*23–25*). The carboxy-terminal region of ICP27 could not replace the transactivating function of the corresponding region of ORF4, and the carboxy-terminal region of ORF4 could not replace the transactivating function of the corresponding ICP27 region. Furthermore, the carboxy-terminal region of ORF4 could not rescue the replication defect of an ICP27 mutant. These findings indicate that while the amino acid sequences of the carboxy-terminal regions are highly conserved, these regions are not interchangeable and require the context of their native proteins.

HSV-1 ICP27 has both transrepressing and transactivating properties in transient expression assays. However, homologs of ICP27 including VZV ORF4, EHV-1 UL3 (*32*), human cytomegalovirus UL69 (*35*), Epstein–Barr virus BMLF1 (*33*), and herpesvirus saimiri ORF57 (*34*) proteins have all been shown to have transactivating but not transrepressing activity. Previous studies indicated that both the acidic amino-terminal and cysteine-rich carboxy-terminal regions are critical for transrepressing activity of ICP27 (*23–26*). We found that the amino- and

carboxy-terminal regions of VZV ORF4 were able to substitute for the corresponding regions in ICP27 to mediate transrepression. These findings suggest that ICP27 contains an additional region that is required for transrepression that is not present in ORF4 or its herpesvirus homologs.

The present data provide additional evidence for the limitation of comparing primary amino acid sequences in predicting functional homologs of alphaherpesvirus proteins. While HSV-1 ICP0 and VZV ORF61 have limited amino acid sequence similarity, and functional homology was not suspected based on initial comparisons of the sequences (*1*), these two proteins can complement each other in functional assays (*13*). In contrast, while HSV-1 ICP27 and VZV ORF4 have marked conservation of their amino acid sequences and were readily identified as potential homologs (*1*), these two proteins cannot complement each other (*17*; this study). Indeed the most conserved portions of these proteins (the cysteine-rich carboxy-termini) do not complement each other, while their less conserved (acidic) amino-termini are complementary in several assays. These findings underscore the importance of continued study of the individual herpesvirus transregulatory proteins.

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