Characterization of the varicella-zoster virus gene 61 protein

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The protein predicted to be encoded by varicella-zoster virus (VZV) gene 61 exhibits limited amino acid sequence similarity to the herpes simplex virus type 1 nuclear phosphoprotein Vmw110, which functions as a transcriptional activator. The gene 61 protein was expressed in its entirety, or as an amino- or carboxy-terminal fragment in *Escherichia coli* and vaccinia virus recombinants, and monospecific rabbit antisera were raised against an *E. coli* fusion between β-galactosidase and the majority of the gene 61 protein. Use of the antisera showed that the gene 61 protein is present in VZV-infected cell nuclei as a heterogeneous phospho-protein of Mr 62K to 65K. Phosphorylation occurs in the amino- and, to a lesser extent, carboxy-terminal portions of the protein. The carboxy-terminal region directs transport of the protein to the nucleus, whereas the amino-terminal region, which contains a potential zinc-binding domain, is responsible for a punctate distribution. Preliminary mapping data indicated that gene 61 is transcribed as a 1-8 kb mRNA which initiates about 65 bp upstream from the translation initiation codon, at a position located appropriately with respect to potential regulatory elements.

Introduction

The varicella-zoster virus (VZV) genome has been predicted to contain 67 distinct genes, 62 of which have counterparts in herpes simplex virus type 1 (HSV-1) based on amino acid sequence conservation (Davison & Scott, 1986; McGeoch *et al.*, 1988). Four of the five HSV-1 immediate early genes have homologues in VZV: those encoding Vmw175, Vmw63, Vmw68 and Vmw110. Vmw175 is an essential protein with a central role in regulation of the HSV-1 transcriptional programme, and has been the subject of intense study (reviewed by Everett, 1987). The VZV gene 62 protein is highly similar (Davison & Scott, 1985; McGeoch *et al.*, 1986) and functionally analogous to Vmw175 (Felser *et al.*, 1988; Disney & Everett, 1990), and has been identified immunologically in infected cells (Felser *et al.*, 1988; Forghani *et al.*, 1990). Vmw63 is an essential protein which regulates later phases of HSV-1 transcription (reviewed by Sandri-Goldin, 1991). The VZV gene 4 protein shows limited similarity to Vmw63 (Davison & Scott, 1986), and has been implicated in transcriptional regulation by transient expression studies (Inchauspe *et al.*, 1989). The VZV gene 63 protein is the counterpart of Vmw68 (Davison & Scott, 1985), which affects the host range of HSV-1 in cell culture (Sears *et al.*, 1985).

Vmw110 is translated from a spliced mRNA that is specified by a gene containing two introns in the protein-encoding region (Perry *et al.*, 1986). The gene is not essential for virus growth in cell culture, but deletion mutants are severely impaired at low m.o.i. (Stow & Stow, 1986). Vmw110 has been shown to activate transcription from several HSV-1 promoters, either alone or in conjunction with Vmw175 (reviewed by Everett, 1987). The protein is phosphorylated (Ackermann *et al.*, 1984) and located in the nuclei of infected cells (Pereira *et al.*, 1977). It has a predicted Mr, of 78452, but migrates with an apparent Mr, of 110000 on SDS-PAGE (Preston *et al.*, 1978). The VZV gene 61 protein (henceforth referred to as 61) has a predicted Mr, of 50913 (Davison & Scott, 1986) and shows a marginal degree of similarity to Vmw110 in a cysteine-rich region which has been proposed to be a zinc-binding domain (Perry *et al.*, 1986). This region is present at residues 116 to 156 of Vmw110 and 19 to 57 of 61, and is also a feature of several eukaryotic proteins (Freemont *et al.*, 1991). Thus, it is quite possible that the two proteins share some functional aspects, but differ in others. Indeed, Inchauspe *et al.* (1989) have indicated that 61 may function in transcriptional regulation, but as a repressor rather than as an activator. In this paper, we have characterized the mRNA and protein products of gene 61 using the techniques of nucleic acid mapping, expression in heterologous systems and antibody studies.

Methods

*Cells and viruses.* Monolayers of CV-1 cells were grown in 5% CO₂ at 37 °C in Dulbecco's modification of Eagle's medium supplemented with 4 mm-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin.
and 5% (v/v) foetal calf serum (FCS). The VZV strain described by Dumas et al. (1981) was passaged in CV-1 cells by trypsinating infected monolayers showing 20 to 50%, c.p.e. and adding the cells at a ratio of 1:5 to 1:1 to unaffected subconfluent monolayers. Infected monolayers were incubated in 5% CO₂ at 37°C until 20 to 50% c.p.e. was observed (usually 1 to 2 days), and were then used for extraction of proteins or RNA, or for immunofluorescence. Vaccinia virus strain WR and recombinants derived therefrom were grown in CV-1 cells. Proteins or RNA were extracted or immunofluorescence was performed 16 to 20 h after infection.

Construction of plasmids. Restriction endonuclease sites used for cloning are indicated on the DNA sequence shown in Fig. 1. VZV fragments were prepared from a plasmid containing VZV KpnI fragment i (Davison & Scott, 1983).

pDK1 was constructed for expression of 61 in Escherichia coli by ligating a fragment extending from the Neol site to the HindIII site downstream from the 3' end of the gene into the vector pUR290 (Rüther & Müller-Hill, 1983) cut with BamHI and PstI.

pDK11 was constructed for expression of 61 in vaccinia virus by inserting VZV KpnI fragment i into the HindIII site of the HindIII site into the vector pKK233-2 consisting of the Neol site encoding residue 1 to the HindIII site into the vector pUR290 (Rüther & Müller-Hill, 1983) cut with BamHI and PstI. Sequences between the HindIII site downstream from gene 61 and the HindIII site in the vector were removed by digesting with HindIII and religating. Sequences between the upstream SaI site in the vector and the Neol site encoding residue 1 were removed from the resulting plasmid by digesting with SaI and (partially with) Neol, repairing the ends and religating. pMJ523 was constructed for expression of 61C in a similar manner by removal of sequences upstream from the internal SaI site. pMJ537 was constructed for expression of 61N by removal of the sequence downstream from the internal SaI site and lacZ under the control of an early promoter. Sequences between the HindIII site downstream from gene 61 and the HindIII site in the vector were removed by digesting with HindIII and religating. Sequences between the upstream SaI site in the vector and the Neol site encoding residue 1 were removed from the resulting plasmid by digesting with SaI and (partially with) Neol, repairing the ends and religating. pMJ523 was constructed for expression of 61C in a similar manner by removal of sequences upstream from the internal SaI site. pMJ537 was constructed for expression of 61N by removal of the sequence downstream from the internal SaI site and lacZ site in the vector.

Construction of vaccinia virus recombinants. Recombinants expressing 61, 61N and 61C at late infection were prepared as described by Davison & Moss (1989). They are denoted VV61, VV61N and VV61C, respectively.

Preparation of rabbit antiserum against 61. The β-galactosidase fusion protein was extracted from bacteria containing pDK1 following induction during the mid-log phase of growth by using 1 mM IPTG for 2 h at 37°C. The protein was purified using the method described by Steers et al. (1971), except that p-aminobenzyl-thio-β-D-galactopyranoside–agarose (Sigma) was employed for affinity chromatography;
1.5 mg protein was obtained per 1 of bacterial culture, but only about 10% was intact as judged by SDS-PAGE. This proportion was recovered even in the presence of several protease inhibitors.

Antisera against the purified fusion protein were prepared by Serotech Ltd., Oxford, U.K. Two sandy halflop rabbits were initially injected subcutaneously with 0.8 mg fusion protein in 0.5 ml emulsified with an equal volume of Freund’s complete adjuvant. The rabbits were boosted on days 28, 56 and 98 with 0.8 mg fusion protein in 0.5 ml emulsified with an equal volume of Freund’s incomplete adjuvant. The rabbits were recovered even in the presence of several protease inhibitors.

Serotec Ltd., Oxford, UK. Two sandy halflop rabbits were initially injected subcutaneously with 0.8 mg fusion protein in 0.5 ml emulsified with 100 μCi/ml L-[35S]methionine and incubated at 30 °C for 16 h in the presence or absence of 1 μg intestinal phosphatase. Aliquots were subjected to immunoprecipitation or immunoblotting.

**Immunoblotting.** Infected cell monolayers were rinsed twice with cold PBS (170 mM-NaCl, 3.4 mM-KCl, 10 mM-Na2HPO4, 1.8 mM-KH2PO4, pH 7.2) and scraped into 1 ml PBS. Cells were pelleted by brief centrifugation in a benchtop microfuge at 4 °C. The nuclear suspension was centrifuged at 3000 r.p.m, for 10 min at 4 °C and the pellet was resuspended in 333 μl buffer C (as buffer B, except that the NaCl concentration was 400 mM). After rotary mixing for 10 min at 4 °C, the suspension was centrifuged at 5000 r.p.m. for 10 min at 4 °C. The pellet was resuspended in buffer D (as buffer B, except that the NaCl concentration was 2 M), sonicated for 10 s at 0 °C in a Heat Systems-Ultrasonics W-380 cup-horn sonicator and centrifuged in a bench microfuge at 13000 r.p.m. for 20 min at 4 °C. The pellet was washed twice with 1 ml buffer B. Supernatants from each step were dialysed overnight at 4 °C against 500 ml buffer B with one change of buffer. Aliquots were heated with boiling mix for SDS-PAGE, and the remainder of each fraction was stored at −20 °C.

**Immunofluorescence.** Cell monolayers were fixed and permeabilized as described by Randall & Dinwoodie (1986). Incubation with antisera and fluorescein isothiocyanate-conjugated goat anti-rabbit antibody (Sigma) was carried out at dilutions of 1:20 and 1:80, respectively. Slides were viewed using a Zeiss Axiosplan Universal microscope and photographed using a Zeiss MC100 camera with Kodak Tri-X Pan 400 film.

**Analysis of RNA.** Total cellular RNA was isolated from uninfected or VZV-infected cells using the acid guanidinium thiocyanate–phenol–chloroform extraction procedure described by Chomczynski & Sacchi (1987). Northern blotting of RNA electrophoresed on formaldehyde–agarose denaturing gels was carried out as described by Sambrook et al. (1989). Nitrocellulose sheets were probed with plasmids radio labelled by nick translation. The 5' end of gene 61 mRNA was mapped by primer extension (Sambrook et al., 1989) of the complementary S' 32P-labelled oligodeoxynucleotide indicated in Fig. 1.

**Results**

**Expression of 61 by VZV and in heterologous systems**

Antisera obtained from rabbits immunized with the major part of 61 fused to β-galactosidase were assayed by immunoblotting of 61 expressed in E. coli and in CV-1 cells infected with VZV or the vaccinia virus recombinant VV61. Results obtained using antiserum obtained at day 125 from one rabbit are shown in Fig. 2. Immune serum from the other rabbit also reacted with 61, but preimmune sera from either rabbit did not (data not shown). The anti-61 serum did not react strongly with proteins in uninduced E. coli containing pKK240-11 (lane 1), but did recognize the high M, β-galactosidase
Fig. 2. Autoradiograph showing results of immunoblotting gene 61 proteins with the anti-61 serum. Lane 1, uninduced E. coli containing pKK240-11; lane 2, IPTG-induced E. coli containing pKK240-11; lane 3, induced E. coli containing pDK11 (expressing 61); lane 4, VZV-infected cells; lane 5, VV61-infected cells; lane 6, uninfected CV-1 cells; lane 7, induced E. coli containing pDK12 (expressing 61N); lane 8, VV61N-infected cells; lane 9, induced E. coli containing pDK13 (expressing 61C); lane 10, VV61C-infected cells. Lanes contained various amounts of extract. Mr markers are shown to the left and in the unlabelled lane.

Fig. 3. Autoradiographs showing results of immunoprecipitation of VZV-infected cell extracts labelled with (a) $^{35}$S or (b) $^{32}$P. Lanes 1 and 3 contain uninfected cell extracts, and lanes 2 and 4 contain VZV-infected cell extracts. Lanes 1 and 2 represent immunoprecipitation using (a) preimmune serum or (b) non-specific rabbit serum, and lanes 3 and 4 immunoprecipitation using anti-61 serum. The heterogeneous set of proteins precipitated by anti-61 serum is indicated to the right of each panel. Mr markers are shown to the left of each panel.

Fragment synthesized in IPTG-induced E. coli containing pKK240-11 (lane 2). When pDK11 was induced, several additional proteins, presumably representing gene 61 translation products, were detected; the largest had an Mr of 62000 (lane 3). In contrast, the antiserum reacted with a range of proteins present in cells infected with VZV or VV61 (lanes 4 and 5) which were absent from uninfected cells (lane 6). The apparently lower Mr of 61 expressed by VV61 was due to an imperfection in the gel; other experiments indicated comparable mobilities (data not shown). The most abundant forms of 61 had MrS of 62K to 65K, but species with MrS in excess of 70000 were evident. The antiserum also recognized β-galactosidase in VV61-infected cells, visible as a high Mr protein in lane 5 because the recombinant contains lacZ in addition to gene 61. The largest 61 species produced in E. coli approximately corresponded in size to the smallest produced in CV-1 cells. Smaller E. coli proteins may have resulted from proteolysis. These results indicate that when synthesized in eukaryotic cells 61 may undergo post-translational modifications which increase its apparent Mr and generate size heterogeneity.

Fig. 2 also shows the results of expressing the amino- and carboxy-terminal portions of 61 (61N and 61C) in E. coli and vaccinia virus recombinants. E. coli 61N was synthesized as a single species with an Mr of 36000 (lane 7; the artefactual bands with slightly higher and lower MrS were not detected in other experiments), whereas proteins produced by VV61N had MrS of 36K to 39K (lane 8). However, direct comparison of size was complicated by the fact that the E. coli and VV61N proteins differ at their carboxy termini; the former is eight amino acid residues longer. There was no difference in mobility between 61C produced in E. coli or by VV61C (lanes 9 and 10). The proteins of higher Mr in these lanes are related to β-galactosidase and its breakdown products, and are particularly noticeable owing to the amount of extract loaded.

Phosphorylation of 61 in vivo

Since Vmw110 is a phosphoprotein and expression of 61 in heterologous systems suggested that it undergoes post-translational modification in eukaryotic cells, immunoprecipitation of cell extracts labelled with $^{35}$S or $^{32}$P was performed. Immunoprecipitation of extracts from $^{35}$S-
labelled VZV-infected cells showed recognition of a heterogeneous set of proteins by the anti-61 serum. The most abundant migrated with \( M_r \)s in the region of 62K to 65K, but larger species were again detected (Fig. 3a, lane 4). Proteins of similar size were precipitated from \(^{32}\)P-labelled extracts (Fig. 3b, lane 4), but not by a non-specific rabbit antiserum (lane 2). These results indicate that 61 is present as a phosphoprotein in VZV-infected cells. Rather weak reactions were observed consistently, and presumably reflect low levels of 61 present in the extracts or low affinity of the antiserum in immunoprecipitation reactions.

\(^{35}\)S-labelled proteins were also specifically precipitated from cells infected with VV61, VV61N and VV61C, and these corresponded in \( M_r \) to proteins detected by immunoblotting (62K to 65K, 36K to 39K and 30K, respectively; Fig. 4a, lanes 2 to 4). Each of these proteins was shown to be phosphorylated (Fig. 4b, lanes 2 to 4), indicating that phosphate groups are present in at least two regions of 61. The amount of extract loaded for different recombinants cannot be considered to be equivalent, because different proportions of cells were detached from monolayers during infection. It appears, for example, that more cells were lost after infection with VV61N. Nevertheless, because samples of the same

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**Fig. 4.** Autoradiographs showing results of immunoprecipitation with the anti-61 serum of extracts from cells infected with vaccinia virus recombinants and labelled with (a) \(^{35}\)S or (b) \(^{32}\)P. Lanes 1, uninfected cells; lanes 2, VV61-infected cells; lanes 3, VV61N-infected cells; lanes 4, VV61C-infected cells. Specifically precipitated proteins related to 61 are indicated by vertical lines to the right of each lane. \( M_r \) markers are shown to the left of each panel.

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**Fig. 5.** Autoradiographs showing the result of treating extracts of cells infected by vaccinia virus recombinants with calf intestinal phosphatase. (a) Immunoprecipitation of \(^{32}\)P-labelled extracts; (b) immunoblotting of \(^{35}\)S-labelled extracts with anti-61 serum. Lanes 1 and 2, VV61-infected cells; lanes 3 and 4, VV61N-infected cells; lanes 5 and 6, VV61C-infected cells; lanes 7 and 8, uninfected cells. Samples in lanes 2, 4, 6 and 8 were treated with calf intestinal phosphatase. Specifically recognized proteins are indicated to the right of each pair of lanes. \( M_r \) markers are shown to the left of each panel.
volume were loaded on corresponding lanes in Fig. 4(a) and (b), the relative intensities of the $^{32}\text{P}$- and $^{35}\text{S}$-labelled proteins indicate that 61N is much more highly phosphorylated than 61C. Therefore, it is likely that 61 is phosphorylated at multiple sites, the majority in the amino-terminal region.

**Dephosphorylation of 61 in vitro**

To determine whether phosphorylation contributes to the greater $M_r$ of 61 expressed in eukaryotic cells and to its size heterogeneity, $^{35}\text{S}$- and $^{32}\text{P}$-labelled extracts from cells infected with vaccinia virus recombinants were dephosphorylated using calf intestinal phosphatase and analysed by immunoprecipitation (Fig. 5a) and immunoblotting (Fig. 5b). The $M_r$ of 61 was reduced by dephosphorylation, although only a proportion of phosphate groups were removed and a degree of size heterogeneity was retained (Fig. 5a, lanes 1 and 2). In this experiment only a small amount of phosphorylated 61N was detected before treatment, and the label was not detected after dephosphorylation (Fig. 5a, lanes 3 and 4).
In contrast, phosphate groups on 61C were refractory, and the $M_r$ was not altered (Fig. 5a, lanes 5 and 6). These results were confirmed by immunoblotting experiments, in which the $M_r$s of major forms of 61, 61N and 61C after treatment with phosphatase were 62000, 36000 and 30000, respectively (Fig. 5b, lanes 2, 4, 6). The proportion of 61N in the higher $M_r$ form (39000) prior to dephosphorylation was greater in most other experiments (data not shown). These results show that the size heterogeneity of 61 is due to phosphorylation in the amino-terminal region, and that phosphoamino acids in this region are more vulnerable to calf intestinal phosphatase than those in the carboxy-terminal region.

The stronger reaction of the antiserum in immunoblots with dephosphorylated forms of 61 and 61N was noted in several experiments. This probably reflects the fact that the antisera were raised against a portion of 61 expressed in *E. coli*, in which 61 does not undergo processing similar to that which occurs in eukaryotic cells. Thus, phosphorylation of 61 in eukaryotic cells may mask antigenic sites present on the *E. coli* protein. Also, since antibodies are directed against only a portion of 61, it is likely that the region from residues 105 to 230 contains at least some of the amino acids whose phosphorylation results in the observed increase in $M_r$.

Subcellular localization of 61

Immunofluorescence experiments showed that 61 is located in a punctate pattern in the nuclei of cells infected with VV61 (Fig. 6a). In VZV-infected cells, 61 was also detected in the nuclei, but in a more diffuse pattern (Fig. 6b). 61 is apparently absent from nucleoli in VZV-infected cells, and this is probably also the case for VV61-infected cells. Fig. 6(b) also shows several uninfected CV-1 cells. 61N was detected in the cytoplasm (Fig. 6c), whereas 61C was located in a diffuse pattern in the nuclei (Fig. 6d). Since the anti-61 serum was raised against a $\beta$-galactosidase fusion protein, expression of $\beta$-galactosidase by the vaccinia virus recombinants presented a potential problem in these experiments. However, the levels of interference by antibodies against $\beta$-galactosidase were shown to be minimal by the use of antisera prepared against other $\beta$-galactosidase fusion proteins and vaccinia virus recombinants expressing $\beta$-galactosidase, but not 61 (data not shown).

The results of immunofluorescence were confirmed by subcellular fractionation experiments (Fig. 7). 61 was detected in the nuclei of cells infected with VV61, and was not eluted by sonication in the presence of $2M$-NaCl (Fig. 7a). 61N was detected in the cytoplasmic fraction (Fig. 7b), but 61C was largely resistant to extraction from nuclei, only a minor proportion being solubilized in $2M$-NaCl (Fig. 7c). In each case, $\beta$-galactosidase expressed by the recombinants was present in the cytoplasm. It is possible that the relative insolubility of nuclear 61 and 61C results from high levels of expression by the vaccinia virus recombinants. Attempts to detect 61 in fractionated VZV-infected cells were unsuccessful, presumably owing to low levels of antigen.
Preliminary mapping of the gene 61 mRNA

A Northern blot of total cellular RNA extracted from uninfected or VZV-infected cells was probed with pDK1 (Fig. 8a). A single VZV-specific RNA species of approximately 1.8 kb was detected (lane 2). It was not detected when the parent plasmid, pUR290, was used as probe (data not shown). Primer extension of an oligodeoxyribonucleotide complementary to the proposed gene 61 mRNA (see Fig. 1) generated a product of approximately 70 nucleotides specifically from VZV-infected cell RNA (Fig. 8b, lane 2). Thus, the proposed 5' end of gene 61 mRNA is located about 65 bp upstream from the translation initiation site (see Fig. 1). The size of the transcript is consistent with polyadenylation in the region predicted from sequence considerations, close downstream from the ATTAAA element shown in Fig. 1 (Davison & Scott, 1986).

Discussion

The existence of gene 61 was predicted from DNA sequence data on the basis of the presence of a leftward-oriented open reading frame containing 467 codons in the genome (Davison & Scott, 1986). We have confirmed the presence of this gene by showing that it is transcribed and translated in VZV-infected cells. We have also characterized the gene 61 protein expressed by VZV and vaccinia virus recombinants and in E. coli.

Full-length 61 expressed in E. coli has an $M_r$ of 62,000, a size which fits well with that reported for 61 synthesized by transcription and translation in vitro (Forghani et al., 1990). In contrast, the protein expressed by VZV or VV61 is larger and has a heterogeneous $M_r$ of 62K to 65K. The difference in $M_r$ between proteins expressed in prokaryotic and eukaryotic cells is due, at least largely, to phosphorylation at multiple sites, particularly in the amino-terminal region.

The protein is located in the nuclei of VZV- and VV61-infected cells. It is present in the latter in a distinct punctate distribution, whereas in the former the pattern is more diffuse. Similar results have been reported for Vmw110, which gave punctate nuclear fluorescence when expressed by transfection of the Vmw110 gene, but a more diffuse fluorescence when expressed in conjunction with Vmw175 (Gelman & Silverstein, 1986). When expressed by recombinant vaccinia viruses, 61C gives a non-punctate nuclear fluorescence, whereas 61N remains in the cytoplasm. Therefore, sequences in the carboxy-terminal region of 61 are responsible for transport into or retention in the nucleus, and sequences in the amino-terminal region, which contains the potential zinc-binding domain, are required for punctate localization. The region responsible for nuclear localization of Vmw110 expressed transiently is also located towards the carboxy terminus (Everett, 1988).

The similarities between 61 and Vmw110 are in accord with the view that the two proteins may share some functional properties. The role of Vmw110 as a transcriptional activator in HSV-1 infection has been investigated in detail (reviewed by Everett et al., 1991). It may act either on its own or in conjunction with Vmw175, the trans-activating ability of which it enhances significantly. Inchauspe et al. (1989) have found in transfection experiments that a plasmid containing VZV genes
60 and 61 reduces expression of a marker gene (gene 36) from its basal level or from enhanced levels induced by the gene 62 and gene 4 proteins. Since there is no expectation or evidence that the HSV-1 counterpart of the gene 60 protein is involved in transcriptional regulation, these authors have suggested a potential role for 61 in repression of VZV transcription. In contrast, Cabirac et al. (1990) have found that 61 had no effect on expression of gene 36 or its HSV-1 counterpart, UL23, either alone or in conjunction with Vmw175 or the VZV gene 62 protein. These potentially conflicting results may be due to differences between experimental systems, particularly in the DNA fragments used as reporter promoters. More extensive investigations should resolve the confusion, and elucidate the functions of 61 in VZV infection.

Gene 61 is transcribed in VZV-infected cells to give a 1.8 kb RNA. This is consistent with the results of Reinhold et al. (1988), who have mapped an appropriately oriented 1.8 kb RNA to the region of the genome containing genes 59, 60 and 61 by Northern blot analysis. Our transcript mapping data are consistent with initiation and polyadenylation of an unspliced mRNA of this size at the positions indicated in Fig. 1. Since, however, the Vmw110 mRNA is spliced, our data must be considered as preliminary because we have not systematically examined whether splicing occurs in transcription of gene 61. Nevertheless, we have noted in Fig. 1 that the proposed 5' end of the mRNA maps about 25 bp downstream from an excellent candidate TATA element, which is downstream from two potential CAAT sequences.

The Vmw110 gene possesses several upstream elements essential for immediate early expression, such as the TAATGARAT sequence (Mackem & Roizman, 1982). We examined carefully the region upstream of gene 61, but were unable to identify convincing examples of such elements. Thus, there is no indication from sequence data that gene 61 is an immediate early gene. Kinetic analysis of VZV gene expression is problematic because high titre cell-free virus of a satisfactory infectivity ratio cannot be prepared in cell culture. The two studies that have been carried out have identified different, but possibly overlapping, sets of immediate early proteins (Lopetegui et al., 1985; Shiraki & Hyman, 1987). It is not clear whether any of these proteins correspond to 61. The possibility that gene 61 is not an immediate early gene has precedents. The counterpart of gene 61 in a related herpesvirus, pseudorabies virus, is an early gene (Cheung, 1991), and transcription of the gene 61 homologue in equine herpesvirus 1 has not been detected under immediate early conditions (Gray et al., 1987; E. Telford, M. Watson, K. McBride & A. Davison, unpublished data). In these respects pseudorabies virus and equine herpesvirus 1, each of which has a single immediate early gene (that encoding the counterpart of Vmw175: Fenwick & McMenamin, 1984; Gray et al., 1987), are likely to be better analogues of VZV than is HSV-1.

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