VP22 is a major tegument protein of herpes simplex virus type 1 and is highly phosphorylated in the infected cell. Indirect evidence exists to suggest that it is encoded by gene UL49, present in the BamHI F fragment of the genome. Using the polymerase chain reaction we have cloned the UL49 open reading frame into a mammalian expression vector under the control of the human cytomegalovirus immediate early gene promoter. After transfection into COS-7 cells expression of the gene product was detected by means of Western blotting and immunofluorescence. The results clearly indicate that the protein encoded by UL49 is VP22, and that in transfected cells it appears to have characteristics similar to those of the protein synthesized in infected cells.

The herpes simplex virus type 1 (HSV-1) protein VP22 is a structural component of the virion and is located in the tegument of the virus particle, along with three other major proteins, VP1/2, VP13/14 and VP16 (Spear & Roizman, 1972). VP22 has been identified as a basic, highly phosphorylated protein which is present in the nuclear fraction of infected cells as well as virions (Halliburton, 1972; Gibson & Roizman, 1974; Knopf & Kaerner, 1980). Initial characterization led to the suggestion that VP22 might be a processed form of protein VP22a, which is present in empty nucleocapsids (Gibson & Roizman, 1974), but it has since been shown that these proteins are not related (Rixon et al., 1988). The function of VP22 and whether it is essential for growth of HSV-1 are yet to be determined, but there is evidence to suggest that it may associate with the nuclear matrix during the replication cycle (Pinard et al., 1987).

VP22 exhibits polymorphism in $M_r$, being 38K in HSV-1 and 36K in HSV-2 (Marsden et al., 1978). This has been utilized in the study of intertypic recombinants between the two serotypes to map the position of the gene encoding VP22 to a region between map positions 0.65 and 0.7 on the HSV-1 genome (Marsden et al., 1978). The DNA sequence of this region (McGeoch et al., 1988) contains a cluster of four genes without introns, two of which have been assigned protein products, namely the UL47 gene, which encodes the tegument protein VP13/14 (McLean et al., 1990; Whittaker et al., 1991), and UL48, which encodes VP16, the α trans-activating protein (Dalrymple et al., 1985). The UL46 gene encodes an unidentified protein with a predicted size of 78K. The UL49 gene encodes a polypeptide with a predicted size of 33K (McGeoch et al., 1988) which is rich in arginine and lysine residues, and thus represents the likely candidate for the gene encoding the basic protein VP22. This report describes the cloning of UL49 by use of the polymerase chain reaction (PCR) into a eukaryotic expression vector, and identification of the gene product expressed in COS-7 cells as VP22. A monoclonal antibody (MAb) directed against VP22 was used to study the expressed protein by Western blot analysis, and to investigate the cellular location of the protein in transfected as well as virus-infected cells.

Fig. 1(a) shows $[^{35}S]$methionine- and $[^{32}P]$orthophosphate-labelled polypeptide profiles of HSV-1- (lane 1) and HSV-2- (lane 2) infected BHK cells. The variation in the Mr of VP22 is evident, and the $^{32}P$-labelled profiles indicate that VP22 is highly phosphorylated in infected cells. MAb P43, which was isolated following immunization of mice with a salt extract of purified HSV-1 proteins containing mainly VP13/14, VP16 and VP22 (Meredith et al., 1991), precipitates a 38K phosphoprotein from HSV-1-infected cells (data not shown), and reacts with the equivalent 38K and 36K proteins of purified HSV-1 and HSV-2 on Western blots (Fig. 1b).

The UL49 gene of HSV-1 strain 17 was amplified by PCR and cloned as shown in Fig. 2. Using the published sequence (McGeoch et al., 1988), primers were designed which hybridized at the exact 5′ and 3′ ends of the UL49 open reading frame (ORF) (Fig. 2a), with a BglII site being incorporated at the extreme 5′ end of each primer to ease manipulation of the amplified fragment. Amplification from HSV-1 genomic DNA was carried out using the Perkin-Elmer Cetus Gene-Amp kit according to the
Fig. 1. SDS-PAGE analysis of HSV-1- and HSV-2-infected cells. (a) HSV-1- (lane 1), HSV-2- (lane 2) and mock- (lane 3) infected cells were labelled from 6 to 24 h post-infection with 20 μCi/ml [35S]methionine (>800 Ci/mmol) or with 200 μCi/ml [32p]orthophosphate. The positions of HSV-1 VP13/14, VPI6 and VP22 are indicated, with the difference in size of VP22 from HSV-1 and HSV-2 shown by arrowheads. (b) Western blot of HSV-1- (lane 1) and HSV-2- (lane 2) infected cells reacted with MAb P43 (anti-HSV-1 VP22).

The manufacturer’s instructions, except that 20% glycerol was included in the reaction mix. Five cycles of 1 min at 97 °C, 1 min at 55 °C and 2 min at 72 °C, followed by 25 cycles of 1 min at 94 °C, 2 min at 55 °C and 2 min at 72 °C were performed. Upon amplification a specific fragment of the correct size for the ORF (0.9 kb) was obtained, and this was digested and cloned into the BglII site of the vector pGE113 to make pGE109 (see Fig. 2b). pGE113 is derived from the expression vector pKV461 (Adams et al., 1988; generously provided by S. M. Kingsman) which contains the human cytomegalovirus (HCMV) immediate early (IE) gene promoter, and the simian virus 40 (SV40) splice and polyadenylation signals. The SV40 origin of replication (ori), which enables the plasmid to replicate in COS cells, was inserted into pKV461 to produce pGE113.

Plasmid pGE109 DNA was used to transfect COS-7 cells at a concentration of 1 μg/4 × 10^5 cells using the DEAE-dextran/chloroquine method (Cullen, 1987). After exposure to the DNA the cells were incubated for 48 h to allow expression of the protein. At this time the cells were either fixed for immunofluorescence staining,
Fig. 3. Immunofluorescence analysis of the expression of VP22 in COS-7 cells. Coverslip cultures of COS-7 cells were transfected with the control plasmid pGE113 (a), pGE109 (b) or infected with HSV-1 (c). Transfected cells were fixed and stained with MAb P43 and fluorescein-labelled anti-mouse IgG after 48 h. Infected cells were fixed and stained in the same way 6 h after infection.

or solubilized for electrophoresis by SDS-PAGE followed by Western blotting. Fig. 3 shows the results of immunofluorescence of transfected and infected COS cells, carried out using MAb P43. The anti-VP22 antibody reacted strongly with COS cells transfected with pGE109 (Fig. 3b), but not with cells transfected with the control parental plasmid, pGE113 (Fig. 3a). The protein appeared to be localized primarily in the cytoplasm, being highly concentrated around the nuclear membrane. When this staining pattern was compared to that exhibited in cells infected with HSV-1, which were fixed early in infection and treated in an identical manner (Fig. 3c), similarities could be seen. Although it was difficult to identify specific regions of the infected cells as they were beginning to show c.p.e., a large amount of the VP22 in these cells was localized around the nucleus. However, this does not exclude the possibility that the protein moves into the nucleus later in infection.

Western blot analysis of the protein expressed from UL49 is shown in Fig. 4. VP22 produced in transfected cells (lane 2) migrates a distance similar to that synthesized in infected cells (lane 3) and that present in purified virions (lane 4), i.e. with an Mr of around 38K. This would suggest that the protein expressed in transfected cells is an authentic form of the virus protein and, taken together with the immunofluorescence study, provides definitive evidence that gene UL49 encodes virus protein VP22.

It is now evident that a cluster of genes in the HSV-1 genome, UL47 to UL49, encodes a group of proteins which have a similar location in the virion, time of synthesis in the infected cell, and phosphorylation status in both the virion and infected cell. This feature is unusual in the HSV-1 genome; the cluster of glycoprotein genes gE, gI, gD and gG (McGeoch et al., 1985) represents the other known example of genes encoding proteins with similar structural characteristics being located in the same region of the genome. The possibility that VP22 and VP13/14 are transcribed and therefore
translated at the same time as the trans-activator VP16 may indicate that interactions between these proteins are important in the regulation of the virus replication cycle. Evidence already exists to suggest that the interaction between VP16 and VP13/14 is more than that of structural components in the virus particle because the presence of VP13/14 has been shown to modulate the trans-activating function of VP16 (McKnight et al., 1987; Zhang et al., 1991). VP22 has been shown to associate with the nuclear matrix (Pinard et al., 1987), a feature which may imply that it has a role in transcription (reviewed by Cook, 1989) and thus an involvement in the control of gene expression in HSV-1-infected cells.

It will be important to demonstrate that VP22 expressed in transfected COS cells undergoes post-translational modifications similar to those of VP22 present in infected cells before this system can be utilized to investigate the interaction of VP22 with other virus proteins. Apart from the high levels of phosphorylation present on infected cell VP22, the protein has been shown to undergo several other forms of modification. Glycosylation by O-linked carbohydrates has been demonstrated (Meredith et al., 1991), a modification which VP13/14 and RNA polymerase II transcription factors such as SP1, AP1 and CTF (Jackson & Tjian, 1988) also undergo. A virus protein of the same Mr as VP22 has also been demonstrated to undergo poly(ADP-ribosylation) in vitro (Preston & Notarianni, 1983), and although the function of this modification is not clear, it is known to occur on several other viral regulatory proteins (Goldman et al., 1981; Goding et al., 1983). Studies are currently under way to determine the extent of the post-translational modification of VP22 expressed from our plasmid in COS cells. If the transfected protein is modified in the same way as that expressed in infected cells, as well as being localized in the same cellular compartments, then this system may prove to be a valid method of investigating the interaction of VP22 with the other virus tegument proteins.

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References


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