The herpes simplex virus type 1 strain 17 open reading frame RL1 encodes a polypeptide of apparent Mr 37K equivalent to ICP34.5 of herpes simplex virus type 1 strain F

E. M. McKay, B. McVey, H. S. Marsden, S. M. Brown and A. R. MacLean*

MRC Virology Unit, Church Street, Glasgow G11 5JR, U.K.

The region between the ‘a’ sequence and the 5' end of the IE1 gene within the long repeat sequence of the herpes simplex virus (HSV) genome plays an important role in the neurovirulence of both HSV-1 strain F and HSV-1 strain 17. However, there has been controversy over the protein-coding potential of this region. Although an open reading frame (ORF) was predicted in HSV-1(F) and shown to encode a polypeptide called ICP34.5, only recently has a corresponding ORF, designated RL1, been recognized in HSV-1(17). To determine whether the HSV-1(17) ORF is expressed, we raised antipeptide sera against predicted amino acid sequences from RL1; one serum specifically recognized a 37K protein in HSV-1(17)-infected cell extracts. Compared with the corresponding HSV-1(F) polypeptide the HSV-1(17) protein has a lower apparent Mr, shows similar kinetics of accumulation and intracellular localization but may accumulate to lower levels than the HSV-1(F) protein. The non-neurovirulent HSV-1(17) deletion variant 1716 fails to synthesize detectable levels of ICP34.5. Thus we have established that HSV-1(17), like HSV-1(F), expresses ICP34.5, a protein important for HSV neurovirulence.

Several groups have mapped a herpes simplex virus (HSV) neurovirulence locus to the long repeat sequence between the ‘a’ sequence and 5' end of the IE1 gene (Thompson et al., 1983, 1989; Taha et al., 1988a, b; Chou et al., 1990; MacLean et al., 1991). However, there has been controversy over the protein-coding potential of this region. In 1986 Ackermann et al., using an antiserum against a peptide consisting of the trimer proline-alanine-threonine repeated 10 times [(PAT)10] from a predicted open reading frame (ORF) of HSV-1(F), identified a 43K polypeptide, named ICP34.5, whose sequence was subsequently revised (Chou & Roizman, 1990). This polypeptide has subsequently been shown to play a crucial role in HSV-1(F) neurovirulence (Chou et al., 1990). However, at the time there was no identified equivalent ORF in HSV-1(17) (McGeoch et al., 1988; Perry & McGeoch, 1988). Subsequently the published sequence for this HSV-1(17) region has also been amended and shown to encode an ORF, designated RL1, equivalent to the ICP34.5 of HSV-1(F) (McGeoch et al., 1991; Dolan et al., 1992). The number of copies of the PAT repeat varies between strains, being present 10 times in HSV-1(F) giving an ICP34.5 of 263 amino acids in length but only five times in HSV-1(17) giving an ICP34.5 of 248 amino acids.

Our aim was to examine directly whether the HSV-1(17) ORF, RL1, encodes a polypeptide equivalent to HSV-1(F) ICP34.5. As a control the non-neurovirulent HSV-1(17) deletion variant 1716, which has a mutation in RL1, was used.

Antisera were raised against seven different peptides from RL1 (Table 1 and Fig. 1). These antisera were screened by Western blot and immunoprecipitation against both HSV-1(F)- and HSV-1(17)-infected cells extracts. Only two of the peptide antisera recognized an HSV-specific polypeptide (3 and 4, Table 1 and Fig. 1). On Western blots antisera to peptide 3, a branched peptide containing seven repeats of the PAT trimer, reacted weakly at a 1:10 dilution with a 39K HSV-1(F) polypeptide but failed to recognize an HSV-1(17) protein specifically (data not shown).

In contrast, sera raised against a linear peptide containing 10 repeats of the PAT trimer (peptide 4) coupled to a carrier protein, keyhole limpet haemocyanin, reacted strongly (down to a 1:1000 dilution) with a 39K HSV-1(F) polypeptide and weakly with a 37K HSV-1(17) polypeptide (Fig. 2a). This interaction was specific: the protein was not detected in extracts from either mock-infected cells or cells infected with the HSV-1(17) RL1 deletion variant 1716 (Fig. 2a); no reaction was detected using preimmune sera (data not shown); interactions were completely blocked by the addition of...
Table 1. Antipeptide sera raised against different regions of ICP34.5

<table>
<thead>
<tr>
<th>Antipeptide serum</th>
<th>Sequence</th>
<th>Carrier protein</th>
<th>Rabbit strain (number immunized)</th>
<th>Sera tested and result*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>YAARLARRGSSWARE</td>
<td>BSA</td>
<td>Half Sandy Lop (2)</td>
<td>- -</td>
</tr>
<tr>
<td>1B</td>
<td>(YAARLARRGSSWARE)K_A</td>
<td>None</td>
<td>Half Sandy Lop (2)</td>
<td>- -</td>
</tr>
<tr>
<td>2A</td>
<td>YEAVIGPCLGPEAR</td>
<td>BSA</td>
<td>Half Sandy Lop (2)</td>
<td>- -</td>
</tr>
<tr>
<td>2B</td>
<td>(YEAVIGPCLGPEAR)K_A</td>
<td>None</td>
<td>Half Sandy Lop (5)</td>
<td>- -</td>
</tr>
<tr>
<td>3A</td>
<td>[(PAT)_7]K_A</td>
<td>None</td>
<td>New Zealand White (2)</td>
<td>+/ -</td>
</tr>
<tr>
<td>3B</td>
<td>[(PAT)_7]K_A</td>
<td>None</td>
<td>New Zealand White (3)</td>
<td>- -</td>
</tr>
<tr>
<td>4</td>
<td>(PAT)_{10}C</td>
<td>K.LH</td>
<td>New Zealand White (2)</td>
<td>+ +</td>
</tr>
<tr>
<td>5</td>
<td>(RARARALARGAPNSV)K_A</td>
<td>None</td>
<td>New Zealand White</td>
<td>- -</td>
</tr>
<tr>
<td>6</td>
<td>(MARRRRHRGPRPRP)K_A</td>
<td>None</td>
<td>New Zealand White (2)</td>
<td>- ND§</td>
</tr>
<tr>
<td>7</td>
<td>(PGPTGVAPTAEQSVT)K_A</td>
<td>None</td>
<td>New Zealand White (2)</td>
<td>- ND</td>
</tr>
</tbody>
</table>

* Tested in Western blots and immunoprecipitation.
† Faint result, the better of the two sera was positive at 1:10 dilution on Western blot.
‡ This serum recognizes only HSV-I(17) ICP34.5 by Western blot.
§ ND, Not determined.

![Image](image)
Fig. 2. A Western blot of HSV-1-infected cell extracts was carried out. After transfer, the nitrocellulose was blocked for 1 h in PBS containing 5% dried milk and then incubated in a 1:1000 dilution of an antiserum raised against the (PAT)$_8$(Table 1: peptide 4). Detection was carried out using Protein A-peroxidase (Sigma) and chemiluminescence (Amersham). Apparent M$_s$ were determined from a [³⁵S]methionine-labelled HSV-infected cell extract. ICP34.5-related bands are indicated with arrowheads. (a) Lane 1, HSV-1(F); lane 2, HSV-1(17); lane 3, HSV-1(17) variant 1716; lane 4, mock-infected. (b) The specificity of the reaction was demonstrated by peptide inhibition of the antibody reaction. The antiserum was preincubated with either 10 μg of the peptide against which the antiserum was raised (peptide 4) (lanes 2, 4 and 6) or with an unrelated peptide (lanes 1, 3 and 5): HSV-1(F), lanes 1 and 2; HSV-1(17), lanes 3 and 4; HSV-1(17) variant 1716, lanes 5 and 6. Bands specifically inhibited by preincubation with the peptide are marked (●).

presence of phosphonoacetic acid (PAA; an inhibitor of HSV DNA synthesis) were also harvested at 24 h p.i. Samples were analysed by Western blotting. Similar kinetics of accumulation were observed with both HSV-1(F) and HSV-1(17); synthesis was first detected 2 h p.i. and polypeptide levels gradually increased until they reached a plateau by 8 h p.i. At 24 h p.i. the level of ICP34.5 was approximately twofold lower in the presence of PAA than in its absence (Fig. 3). The absence of Vmw21 (a true late polypeptide) (Johnson et al., 1986) confirmed that the PAA blocked HSV DNA synthesis (data not shown). ICP34.5 shows similar kinetics of accumulation to those of gD (Johnson et al., 1986) and can thus be designated a delayed early polypeptide in agreement with the classification of the HSV-1(F) ICP34.5 promoter as $\gamma_1$ by Chou & Roizman (1986).

From the data presented in this paper we have demonstrated that the HSV-1(17) RL1 ORF, located between the 'a' sequence and 5' end of the IE1 gene (Dolan et al., 1992), encodes a polypeptide equivalent to the ICP34.5 of HSV-1(F). The predicted amino acid sequences of the two polypeptides are very similar. Not surprisingly therefore, both proteins showed similar kinetics of accumulation and were localized almost exclusively in the cytoplasm [a low level detected in the nucleus (<1/320) was probably due to cytoplasmic
was further separated into tightly associated membrane proteins (lane 4). To quantitify nucleus-associated ICP34.5 this fraction was concentrated 50-fold (lane 1). Cells were also separated into cytosolic (lane 6) and total membrane-bound fractions (lane 4). The membrane-bound fraction was further separated into tightly associated membrane proteins (lane 5). HSV-1(17) virions were also analysed (lane 7). The ICP34.5 bands are indicated by an arrow.

Fig. 4. A Western blot of ICP34.5 separation into different cellular subfractions from HSV-1(17)-infected cell extracts. Cells were separated into cytoplasmic (lane 2) and nuclear fractions (lane 3). To quantify nucleus-associated ICP34.5 this fraction was concentrated 50-fold (lane 1). Cells were also separated into cytosolic (lane 6) and total membrane-bound fractions (lane 4). The membrane-bound fraction was further separated into tightly associated membrane proteins (lane 5). HSV-1(17) virions were also analysed (lane 7). The ICP34.5 bands are indicated by an arrow.

contamination] and were not associated with membranes (Fig. 4). This cytoplasmic localization was also reported by Ackermann et al. (1986). In addition ICP34.5 was not detectable in virions (Fig. 4). HSV-1(F) ICP34.5 is phosphorylated, and it is also likely that HSV-1(17) ICP34.5 will be a phosphoprotein. As expected, the non-neurovirulent HSV-1(17) variant 1716 (with a deletion removing 90% of the RL1 ORF) fails to synthesize ICP34.5. This demonstrates that, as is the case with HSV-1(F) (Chou et al., 1990), ICP34.5 of HSV-1(17) plays a crucial role in HSV replication in the nervous system (MacLean et al., 1991).

The two main differences between the HSV-1(F) and HSV-1(17) polypeptides are in their apparent Mr's and the level of their detection. The slight decrease in electrophoretic mobility of the HSV-1(F) polypeptide (apparent Mr 39K compared to 37K) is probably due to the presence of 15 extra amino acids. The difference in the level of detection of the HSV-1(F) and HSV-1(17) polypeptides may be due either to weaker antibody affinity for the HSV-1(17) polypeptide or to a lower level of accumulation of the HSV-1(17) polypeptide. Additional antisera will be required to address this question; we are currently attempting to express the HSV-1(17) polypeptide in Escherichia coli to generate an antiserum against the whole protein. It has been shown previously that the level of accumulation of HSV polypeptides may vary considerably between strains (Marsden et al., 1976, 1978), but if there is a difference in the level of accumulation of ICP34.5 this does not affect the relative neuropathogenicity of HSV-1(F) and HSV-1(17) (L. Robertson, personal communication).

We would like to thank Dr Christine MacLean and Miss Ania Owsianka for allowing us to incorporate their unpublished data. We would also like to thank Professor J. H. Subak-Sharpe for his overall interest in this work, Dr June Harland for helpful criticisms and Dr Duncan McGeoch for his critical reading of this manuscript. Part of this work was funded by SmithKline Biologicals, Belgium.

References


TAHA, M. Y., CLEMENTS, G. B. & BROWN, S. M. (1989a). A variant of herpes simplex virus type 2 strain HG52 with a 1.5 kb deletion in R, between 0 to 0.02 and 0.81 to 0.83 map units is non-neurovirulent for mice. *Journal of General Virology* **70**, 705–716.


(Received 25 March 1993; Accepted 22 June 1993)