Properties of the Herpes Simplex Virus Type 2 Trans-inducing Factor Vmw65 in Wild-type and Mutant Viruses

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

(Accepted 20 February 1989)

SUMMARY

A temperature-sensitive mutant (ts13) of herpes simplex virus type 2 (HSV-2) has a mutation which causes in vitro thermolability of the virion. This mutation lies within the gene encoding a virion structural protein of Mr 65K which is known to stimulate immediate early transcription (the trans-inducing factor, 65K_TIF). The results presented here show that the structural role of 65K_TIF is essential. The electrophoretic mobility of the 65K_TIF encoded by ts13 and a revertant of ts13 differed from that of the wild-type HSV-2 parent. Two monoclonal antibodies directed against 65K_TIF were shown to react with two different epitopes on this polypeptide, one of which was altered by the mutation in ts13. No differences were observed in the phosphorylation status of 65K_TIF from mutant- and wild-type-infected cells.

A temperature-sensitive (ts) mutant of herpes simplex virus type 2 (HSV-2), ts13, has two unrelated ts mutations in different genes. One of these, which is between 0.12 and 0.21 map units (m.u.) on the HSV-2 genome (Moss et al., 1979), lies within the gene encoding the alkaline DNase activity and specifies an enzyme which is ts both in vivo and in vitro (Francke et al., 1978). The position of this mutation has since been narrowed to between 0.145 and 0.184 m.u. on the HSV-2 genome (H. Moss, unpublished data). The other mutation, which maps between 0.64 and 0.70 m.u. on the HSV-2 genome (Chartrand et al., 1981) renders the virion thermolabile in vitro (Halliburton & Timbury, 1976). Both genes appear to be essential for virus replication (Moss, 1986; Moss et al., 1979).

The region containing the mutation affecting virion thermostability has been shown by analysis of HSV-1/HSV-2 intertypic recombinants to encode a major virion polypeptide of Mr 65K (Marsden et al., 1978). This region has subsequently been sequenced for HSV-1 and a number of open reading frames have been identified (Dalrymple et al., 1985; McGeoch et al., 1988). It is known that a component of the virion stimulates immediate early (IE) gene transcription (Post et al., 1981; Cordingley et al., 1983; Batterson & Roizman, 1983) and it has been shown that IE gene transcription is stimulated by a protein that maps within a 27 kb subfragment of BamHI f. The only complete gene (UL48) within this fragment encodes a polypeptide with a predicted Mr of 54342 (Dalrymple et al., 1985) which migrates on an acrylamide gel with an apparent Mr of 63K (Hall et al., 1982). Insertion of an 8 bp linker into DNA sequences encoding this protein abolished its ability to stimulate IE gene transcription (Campbell et al., 1984). Furthermore, by using hybrid-arrested translation and immunoprecipitation with a monoclonal antibody (MAb), MA1044, which is directed against a 65K virion component, Campbell et al. (1984) were able to demonstrate that UL48 encoded this 65K virion polypeptide. Therefore, they concluded that this protein is the virion component responsible for stimulation of IE gene transcription which has subsequently been designated the trans-inducing factor (65K_TIF) (Marsden et al., 1987). The 65K_TIF maps within the same region as the thermostability lesion of ts13. However, stimulation of IE transcription by the BglII f fragment cloned from ts13 is normal at the non-permissive temperature of 38.5 °C (Dalrymple, 1986). Recently, Ace et al. (1988) have used insertional mutagenesis of this gene to map functional
Fig. 1. Map locations of the ts lesions and genomic structures of tsl3 and derivatives of tsl3 (see text for details of these viruses). The top line is a representation of the long region of the HSV genome comprising the long unique region (UL) and the flanking terminal (TRL) and internal (IRL) inverted repeat regions. The two ts defects of tsl3, the DNase lesion (m.u. 0.145 to 0.184) (H. Moss, unpublished data) and the virion thermostability lesion (m.u. 0.64 to 0.70) (Chartrand et al., 1981) have been mapped within the regions indicated by the open boxes. R13(4-8) (Moss et al., 1979) has reverted for virion thermostability but retained the DNase lesion. The maximum sizes of the HSV-1 inserts in the intertypic recombinants R13(4-8)8-3 (Moss, 1986), R13-2 and R13-4 (Chartrand et al., 1981) are shown by hatched boxes.

Fig. 2. Variation in mobility of the 65K band in cells infected with various HSV strains. Fifty mm plates of BHK-21 cells were mock-infected or infected at 20 p.f.u./cell with the appropriate virus and incubated at 38.5 °C. One h post-infection (p.i.), the cells were washed twice with methionine-free medium containing 5% fetal calf serum and then overlaid with 2 ml of this medium. At 5 h p.i. 150 mCi of [35S]methionine was added and incubation at 38.5 °C was continued until 7 h p.i. The cells were then washed three times with phosphate-buffered saline. Samples were then boiled for 5 min in 50 μg 50 mM-Tris-HCl pH 6.7, 2% SDS, 700 mM-2-mercaptoethanol and 10% glycerol containing bromophenol blue to visualize the dye front. They were then run on a 7.5% acrylamide gel as described by Marsden et al. (1976). Lane 1, R13(4-8)8-3; lane 2, tsl3, lane 3, R13(4-8); lane 4, R13-2; lane 5, HSV-2 wt; lane 6, HSV-1 wt; lane 7, mock-infected. The 65K band is indicated by an arrow.

domains within the 65K TF. They have shown that its trans-inducing function can be separated from the ability to rescue the ts mutation from tsl3. It has also been shown that stimulation of IE gene expression requires the association of 65K TF with one or more cellular proteins via defined sequences upstream of these IE genes (O'Hare & Goding, 1988; Preston et al., 1988). The following experiments were designed to examine the properties of the 65K TF in a number of wild-type and mutant viruses.

BHK-21 C13 cells were used throughout this study and were grown in Eagle's medium supplemented with 10% calf serum and 10% tryptose phosphate broth. The HSV-2 wild-type
Fig. 3. Precipitation of 65K<sub>TTP</sub> with MA1044. Cells were infected at 31 °C or 38.5 °C and labelled as described in the legend to Fig. 2 except that 150 μCi [<sup>35</sup>S]methionine was added at 1 h p.i. and incubation was continued until 6 h p.i. when the cells were harvested. Immunoprecipitations were carried out as described by Palfreyman et al. (1984). (a) Extracts from cells infected and labelled at 38.5 °C. The samples are 1, R13(4-8)-3; 2, ts13; 3, R13(4-8); 4, R13-2; 5, HSV-2 wt; 6, HSV-1 wt; 7, mock-infected. Each lane shows polypeptides present after (i) pre-absorption with control ascites fluid, (ii) precipitation with MA1044 and (iii) precipitation with control ascites fluid. (b) MA1044-precipitated polypeptides run in neighbouring lanes. Samples 1 to 7 are as indicated in (a). Sample 8 is a second example of a plaque isolate representing R13(4-8) rescued with HSV-1 EcoR1 fragment d. (i) Pre-absorbed with control ascites fluid; (ii) precipitated with MA1044. (c) Sample 1 refers to ts13-infected cells incubated at 38.5 °C. The remaining samples were from cells infected and labelled at 31 °C: sample 2, ts13; 3, R13(4-8); 4, R13-2; 5, R13-4; 6, HSV-2 wt; 7, HSV-1 wt; 8, mock-infected. All samples were precipitated with MA1044. For each sample, (i), (ii) and (iii) are as described for (a). The 65K band is indicated by an arrow.
Short communication

Fig. 4. Precipitation of 65K\textsubscript{WIF} by MA1044 and LP1. Samples were prepared as described in the legend to Fig. 3 and are 1, \textit{ts}13; 2, R13(4-8); 3, R13(4-8)-3; 4, \textit{R13}-2; 5, HSV-2 \textit{wt}; 6, HSV-1 \textit{wt}; 7, mock-infected; 8, R13-4. Samples grown at 31 °C: a, polypeptides present after pre-absorption with control ascites fluid; b, polypeptides precipitated by MA1044; c, polypeptides precipitated by LP1. Samples grown at 38.5 °C: a', polypeptides present after pre-absorption with control ascites fluid; b', polypeptides precipitated by MA1044; c', polypeptides precipitated by LP1. The 65K band is indicated by an arrow.

(wt) virus used was the parental strain HG52 which had given rise to \textit{ts}13; the HSV-1 \textit{wt} was strain 17.

During studies on cells infected with either \textit{ts}13 or R13(4-8) [previously referred to as 4-8 (Moss, 1986); a revertant of \textit{ts}13 that grows at 38.5 °C and which has recovered virion thermostability but continues to induce a \textit{ts} DNase activity] it was observed that migration of a polypeptide of around 65K on SDS-polyacrylamide gels differed from that of the \textit{wt} HSV-2 virus suggesting that this protein might be related to the \textit{ts}13 structural defect. Therefore, the polypeptide profiles of cells infected with HSV-1 \textit{wt}, HSV-2 \textit{wt}, \textit{ts}13, R13(4-8) or the intertypic recombinants R13(4-8)-3 (Moss, 1986), R13-2 or R13-4 (Chartrand \textit{et al.}, 1981) and labelled with [\textsuperscript{35}S]methionine were compared. In R13(4-8)-3, the \textit{ts} lesion in the DNase gene of R13(4-8) has been rescued with an HSV-1 DNA fragment (\textit{EcoRI} \textit{d}) and in R13-2 and R13-4 the virion thermostability defect of \textit{ts}13 has been rescued using restriction enzyme fragments of HSV-1 DNA (Chartrand \textit{et al.}, 1981). Fig. 1 describes the genome structures of the viruses used in these experiments. Fig. 2 shows the variation in mobility of the 65K band from cells infected with the various virus stocks; the 65K band induced by HSV-2 \textit{wt} migrates more slowly than that of the HSV-1 \textit{wt} (Marsden \textit{et al.}, 1978). The 65K band of R13-2 comigrates with that of the HSV-1 \textit{wt}. The \textit{ts}13, R13(4-8) and R13(4-8)-3 bands migrate at a similar rate but faster than either the HSV-1 or HSV-2 \textit{wt} bands.

In order to confirm that the protein involved in these mobility shifts is 65K\textsubscript{WIF}, immunoprecipitations were performed using MAb MA1044 which is directed against 65K\textsubscript{WIF}. At 38.5 °C, precipitation of 65K\textsubscript{WIF} (Fig. 3a) was observed with extracts of cells infected with R13(4-8) (lane 3, ii), R13(4-8)-3 (lane 1, ii), R13-2 (lane 4, ii), HSV-1 \textit{wt} (lane 6, ii) and HSV-2 \textit{wt} (lane 5, ii) but not with extracts from \textit{ts}13-infected cells (lane 2, ii). Moreover, by running the precipitated polypeptide samples in neighbouring lanes, it was clear that MA1044 was precipitating a protein of variable mobility (Fig. 3b) and that the mobility pattern resembled...
Fig. 5. Phosphorylation patterns of 65K\textsubscript{TIF}. Fifty mm plates of BHK-21 cells were mock-infected or infected at 10 p.f.u./cell and incubated at 38.5 °C apart from \textit{ts}13-infected cells which were incubated at both 31 °C and 38.5 °C. One h p.i., the cell sheets were washed with phosphate-free medium containing 5% foetal calf serum and then overlaid with 2 ml of the same medium containing 100 μCi [\textsuperscript{32}P]orthophosphate. Incubation was continued at 38.5 °C or 31 °C and cells were harvested at 6 h p.i. The cell sheets were washed very briefly in H\textsubscript{2}O and then harvested in 1 ml H\textsubscript{2}O. To each sample, 0.5 ml DNase/RNase mixture and 0.75 ml 3 × denaturing buffer was added (Marsden \textit{et al.}, 1978) and the samples were boiled for 4 min before SDS-PAGE. Samples are 1, \textit{R}13(4-8)-3; 2, \textit{ts}13; 3, \textit{R}13(4-8); 4, \textit{R}13-2; 5, HSV-2 wt; 6, HSV-1 wt; 7, mock-infected. For each sample a, a’, b, b’ and c, c’ are as described in the legend to Fig. 4. The 65K band is indicated by an arrow.

that of the 65K protein described above. MA1044 also failed to precipitate 65K\textsubscript{TIF} from cells infected with \textit{ts}13 at the permissive temperature of 31 °C (Fig. 3c, lane 2, ii); the inability of MA1044 to recognize \textit{ts}13 65K\textsubscript{TIF} is not therefore directly related to the \textit{ts} behaviour of this mutant. The reappearance of 65K\textsubscript{TIF} in \textit{R}13(4-8) samples and the characteristic mobility shifts of the protein from the various virus strains confirm that the \textit{ts}13 lesion maps within this protein and that this protein therefore functions as an essential virion component.

Extracts of cells infected with each of these viruses were then precipitated with a second MAb (LP1) (McLean \textit{et al.}, 1982) also directed against 65K\textsubscript{TIF} (Marsden \textit{et al.}, 1987). Fig. 4 shows that MAb LP1 precipitated 65K\textsubscript{TIF} from cells infected at 31 °C or 38.5 °C with all virus samples including \textit{ts}13 at both temperatures. The 65K\textsubscript{TIF} precipitated by LP1 from cells infected with either \textit{ts}13 or R13(4-8) comigrated as would have been predicted from infected cell profiles.

This result demonstrates that MAb MA1044 and LP1 react with two different epitopes on 65K\textsubscript{TIF} and that the defect in \textit{ts}13 directly alters the epitope of 65K\textsubscript{TIF} which reacts with MA1044 so that it is no longer recognized.

The 65K structural polypeptide which was mapped to the region of the \textit{ts}13 defect is known to be a phosphoprotein (Marsden \textit{et al.}, 1978). Phosphorylation of the 65K\textsubscript{TIF} patterns from cells infected with the various viruses was examined using MAb MA1044 and LP1 (Fig. 5); with MA1044, phosphorylated bands of 65K were precipitated from cells infected with HSV-1 wt, HSV-2 wt, \textit{R}13(4-8), R13-2, \textit{R}13-4 and \textit{R}13(4-8)-3 and had similar mobilities to the respective
65K bands precipitated in the [35S]methionine-labelled infected cells (Fig. 2). No phosphorylated 65K band was precipitated from ts13-infected cells at either the permissive or non-permissive temperature. These results show that 65K_TIF is a phosphoprotein. With MAb LP1, the phosphorylated 65K_TIF band was precipitated from ts13-infected cells at both 31 °C and 38.5 °C suggesting that the ts lesion is not linked to a defect in phosphorylation of this protein.

Fig. 4 (lanes 6 c and c') shows that LP1 precipitated a second pair of polypeptides of apparent M, 122K in addition to 65K_TIF from HSV-1 wt-infected cells. These bands comigrate with gB (Marsden et al., 1987) and these authors have suggested that either LP1 may recognize an epitope common to the 65K_TIF polypeptide and gB or that there may be an interaction between the two proteins. However, LP1 precipitated only 65K_TIF from HSV-2 wt (Fig. 4, lanes 5 c and c'), which suggests that such an association does not occur in HSV-2. Moreover, when the intertypic recombinants R13-2 and R13-4, which are type 1 for 65K_TIF and type 2 for gB, were examined no gB precipitation was observed (Fig. 4, lane 4 c and lane 8 c'). Furthermore, the second anti-65K_TIF MAb, MA1044, failed to precipitate gB from any of the virus extracts examined [HSV-1 wt, HSV-2 wt, R13-2, R13-4 and R13(4-8)-3] (Fig. 3a). These results argue strongly against any direct association between 65K_TIF and gB, hence recognition of an epitope fortuitously common to the 65K polypeptide and HSV-1 gB by LP1 seems to be a more likely explanation.

It is apparent from Fig. 2 that the R13(4-8) 65K_TIF band comigrates with that of ts13 rather than with that of HSV-2 wt. Although it is conceivable that a second mutation rather than a back mutation is responsible for the phenotypic reversion, it is much more likely that ts13 has two mutations in this gene. The first mutation reduces the size of the protein but does not affect either of its functions. The second directly affects its structural but not its trans-inducing function and has reverted in R13(4-8) altering the epitope recognized by MA1044. The sequence of HSV-1 65K_TIF has been analysed by Dalrymple et al. (1985). By determining the sequence of the comparable HSV-2 wt genomic region and carrying out a sequence comparison between the gene encoding the 65K_TIF in both ts13- and R13(4-8)-infected cells, it should be possible to establish the exact location and nature of the mutation affecting virion thermostability.

I should like to thank Professor J. H. Subak-Sharpe, Dr F. J. Rixon and Dr C. M. Preston for their critical reading of the manuscript. The monoclonal antibody MA1044 was a gift from Dr J. Palfreyman.

REFERENCES


Short communication


(Accepted 6 October 1988)