The Product of Gene US11 of Herpes Simplex Virus Type 1 Is Expressed as a True Late Gene

By P. A. JOHNSON, C. MACLEAN, H. S. MARSDEN, R. G. DALZIEL† AND R. D. EVERETT*

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

(Accepted 6 February 1986)

SUMMARY

The genes of herpes simplex virus type 1 (HSV-1) can be divided into at least three temporally regulated groups termed immediate early (IE), early and late. We have studied in detail the expression of a member of the late class of genes, US11, which encodes a polypeptide of apparent molecular weight 21K. Highly specific and sensitive probes were used to monitor US11 RNA and protein synthesis during HSV-1 infection of tissue culture cells in the presence and absence of phosphonoacetic acid, an inhibitor of viral DNA replication. The results were compared with a similar study of the products of a delayed early gene, US6, encoding glycoprotein D (gD). It was found that the patterns of RNA and protein synthesis from US11 were significantly different to those of gD. US11 products appeared later and accumulated until late in infection, while gD RNA was significantly reduced at late times. In the presence of the inhibitor of DNA synthesis, US11 gene expression was reduced 50- to 100-fold while gD expression was reduced five- to tenfold. We conclude that US11 behaves as a true late gene during HSV-1 infection. However, the use of sensitive assays, which allowed the detection of very low levels of US11 gene products under conditions designed to eliminate DNA replication, brings into question the absolute requirement for DNA replication for the expression of a true late HSV-1 gene. These results are discussed in terms of current models for the regulation of late gene expression.

INTRODUCTION

During lytic infection by herpes simplex virus type 1 (HSV-1), at least three groups of temporally regulated genes have been identified: immediate early (IE), early (E) and late (L) or α, β and γ (Honess & Roizman, 1974, 1975; Swanstrom & Wagner, 1974; Jones & Roizman, 1979; for review, see Wagner, 1985). IE genes are expressed in the absence of de novo protein synthesis (Honess & Roizman, 1974), but their expression is stimulated by a component of the virus particle, Vm175 (Campbell et al., 1984). At least two IE gene products, Vm175 and Vm110, are involved in the activation of early genes (Everett, 1984a; O'Hare & Hayward, 1985; Quinlan & Knipe, 1985). The activation of late genes is poorly understood, but their efficient expression is dependent on viral DNA replication (Jones & Roizman, 1979; Holland et al., 1980; Hall et al., 1982), which indicates that there is at least an indirect requirement for prior IE and early gene expression for late gene transcription. To investigate the regulation of late gene transcription for any gene it is first necessary to establish that the pattern of RNA and protein synthesis corresponds to that expected of a late gene. We shall attempt to define in more detail the properties of a late gene in the Discussion section of this paper, but initially we have worked on the premise that late gene products should first appear in the infected cell late in infection, their synthesis should be markedly dependent on viral DNA synthesis, and their pattern of expression should be different from that of genes of the earlier temporal classes.

†Present address: Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, California 92037, U.S.A.
The late gene we have chosen to study is US11 (McGeoch et al., 1985), which lies in the short unique (Us) sequence of the HSV genome and encodes a protein of 161 amino acids with a total mol. wt. of 17756 (McGeoch et al., 1985) and an apparent mol. wt. on SDS–polyacrylamide gels of 21000 (21K) (Rixon & McGeoch, 1984). This late gene was an attractive candidate for an investigation of late promoter regulation since (i) the sequence of US11 and its upstream region has been determined (Murchie & McGeoch, 1982), (ii) the position of the 5' end of its RNA has been mapped (Rixon & McGeoch, 1984) and (iii) a protein of this apparent molecular weight has been preliminarily classified as one of the latest viral proteins to appear during lytic infection, and subsequently to accumulate in large amounts (Marsden et al., 1976). An additional reason for studying this gene is that it lies in an interesting position in the genome. It is the internal member of a family of three 3' co-terminal genes, and its cap sites lie within the coding region of V<sub>mw</sub>12, encoded by US12, also called IE gene 5 (IE-5). The TR<sub>S</sub>/IR<sub>S</sub> origin of DNA replication is less than 1 kb upstream from US11, adjacent to the IE-5 promoter (Stow & McMonagle, 1983).

We have compared the expression of US11 with that of US6, which encodes glycoprotein D (gD). The gD promoter is activated by IE gene products early in infection (Watson et al., 1983; Everett, 1984b), but since it requires DNA replication for maximal expression it has been termed a delayed early, or β<sub>1</sub>, gene (Johnson & Spear, 1984).

In this paper we have (i) defined the precise location of the 5' end of the US11 RNA, (ii) compared the kinetics of accumulation of RNA and protein synthesis from US11 and US6, and (iii) examined the sensitivity of US11 and US6 to the viral DNA replication inhibitor, phosphonoacetic acid (PAA). This report details the kinetic class of the US11 gene and its established gene product, a 21K polypeptide.

**METHODS**

**Cells.** BHK-21 C13 cells (Maepherson & Stoker, 1962) were used throughout, and maintained in Eagle's medium supplemented with 10% calf serum.

**Viruses.** HSV-1 strain 17 syn<sup>+</sup> (Brown et al., 1973) was used in these studies. The phosphonoacetic acid-resistant mutant PAA<sup>−1</sup> was derived from HSV-1 17 syn<sup>+</sup> (Hay & Subak-Sharpe, 1976), and the mutation has been mapped within the DNA polymerase gene (Crumpacker et al., 1980).

**Infection procedure.** Cell monolayers at 75% confluence were infected for 1 h at a multiplicity of 20 p.f.u., in a volume of 1·5 ml of growth medium on 90 mm Petri dishes (for RNA and DNA extractions) or 0·5 ml on 50 mm dishes (for protein extractions). To inhibit viral DNA synthesis, cells were maintained 1 h before, during and after infection in medium containing 300 μg PAA (Sigma product P 6909) per ml.

**Labelling of infected cells.** Infected cell proteins were labelled with [35S]methionine (Amersham) at 50 μCi/ml in Eagle's medium containing one-fifth the normal concentration of methionine and 2% calf serum. Label was present continuously from 0 h post-adsorption, except in the 0 h samples which were labelled during viral adsorption.

**RNA and DNA isolation.** Total cytoplasmic RNA was prepared at various times after infection, by NP40 lysis of the cells and extensive phenol–chloroform extraction of the supernatant after the nuclei had been pelleted. DNA was extracted from the nuclei of the same cells by lysis in 0·6% SDS in the presence of proteinase K (Boehringer Mannheim, product no. 161 519). The samples were then sequentially extracted with phenol and chloroform, treated with RNase A (100 μg/ml) and further extracted with phenol and chloroform prior to ethanol precipitation.

**S1 mapping.** This was performed as described by Everett (1983), using 5 μg RNA per reaction and 0·02 pmol of probe. The probe for S1 mapping of transcripts from US11 was the appropriate 3' end-labelled separated strand from the TaqI–BamHII fragment of pDG8 (see Fig. 1b). The location of the US11 RNA cap sites was determined by running S1-protected hybrids alongside a G + A DNA sequence ladder (Maxam & Gilbert, 1980), derived from the same probe. gD transcripts were quantified by S1 mapping using a single-stranded 5' end-labelled probe from a BstNI fragment (Us coordinates 5563 to 5831; McGeoch et al., 1985) of pGX48 (see Fig. 1c). S1 gels were analysed by densitometry.

**Dot blots.** Spot hybridizations of DNA samples were performed essentially as described by Kafatos et al. (1979). DNA samples were probed for the presence of viral DNA by hybridization to nick-translationed (Rigby et al., 1977) pGX156, which contains the entire Us sequence of HSV-1 (Everett, 1984a). Dot blot autoradiographs were quantified by densitometry.

**Isolation of labelled proteins.** Cells were lysed in an extraction buffer containing 0·1 M-Tris–HCl pH 8·0, 10% glycerol, 0·5% NP40, 0·5% sodium deoxycholate (BDH, product no. 43035) and 0·2 mM-phenylmethylsulphonyl fluoride (Sigma, product no. P 7626) (Zweig et al., 1980) using 0·1 ml extraction buffer per 2 × 10<sup>6</sup> cells. Extracts
were kept on ice for 1 h and then centrifuged for 5 min at 14000 g in a Beckman microfuge. The clarified supernatant was the extract used for immunoprecipitations.

**Antiserum and monoclonal antibodies.** The antiserum designated 12848 was raised by immunization of a rabbit with a conjugate of bovine serum albumin (BSA) and the peptide NH₂-Tyr-Arg-Thr-Ala-Arg-Gly-Ser-Val (Cambridge Research Biochemicals, Cambridge, U.K.). This sequence represents the carboxy-terminal seven amino acids of 21K, as predicted from the DNA sequence (McGeoch *et al.*, 1984) with an additional amino-terminal tyrosine to facilitate coupling of the oligopeptide to BSA (Bassiri *et al.*, 1979). Anti-BSA antibodies were removed from the immune serum by absorption with BSA. Antipeptide antibodies were detected by solid-phase radioimmunoassay, and their reactivity with the relevant protein was demonstrated by Western blotting analysis. The monoclonal antibody 1892, specific for gD, was isolated and characterized by Dr A. C. Cross in our laboratory.

**Immunoprecipitation.** Immunoprecipitation experiments were carried out essentially as described by Zweig *et al.* (1980). Forty µl of cell extract, 50 µl of a 1:100 dilution of either monoclonal antibody 1892 or an ascites control, and 5 µl of a rabbit anti-mouse immunoglobulin (Cedarlane Laboratories, Hornby, Ontario, Canada) were incubated together for 3 h at 4 °C and then for a further 1 h with 60 µl of a 50% (v/v) suspension of Protein A-Sepharose (Sigma). Proteins subsequently eluted from the pellet were analysed on 5 to 12.5% gradient SDS-polyacrylamide gels (Marsden *et al.*, 1978).

**Western blotting.** Western blotting was carried out essentially as described by Towbin *et al.* (1979) with the following two modifications. First, after transfer of proteins from SDS-polyacrylamide gels to a single large sheet of nitrocellulose, the blocking step was carried out at 65 °C, the optimal temperature for detection of bound protein with this antiserum (H. S. Marsden & M. Murphy, unpublished results). Second, after blocking, the nitrocellulose sheet was placed protein side up and a sheet of Whatman paper (grade 182) was laid on top of it. Ten ml of antiserum 12848, at a 1:20 dilution, was pipetted evenly onto the paper and incubated at 37 °C for 2 h. The nitrocellulose was then washed extensively and further incubated with 100 ml of 125I-labelled Protein A (2 x 10⁵ c.p.m./ml; iodinated by the method of Hunter & Greenwood, 1962).

**Autoradiography.** Radioactive DNA and proteins were detected by autoradiography using Kodak X-Omat XSI film.

**Densitometry and quantification of mRNA and protein.** The amount of US11 and gD transcripts and protein were quantified by densitometry of the autoradiographs using a Joyce-Loebl scanning densitometer and measurement of the area of appropriate peaks using a program, DENS, written by Dr P. Taylor for a DEC PDP 11/44 computer linked to a digitizing two-dimensional tablet. The response of the XSI film was found to be linear to within 10% over the optical density range of the peaks analysed. To determine how the optical density of the peak (corresponding to immunoprecipitated gD or blotted and probed 21K) varied with the amount of gD or 21K present, serial twofold dilutions of antigen were made, probed with the anti-gD or anti-21K antibody as described, and the optical density of the peak was determined. The amount of gD measured was directly proportional to the amount of protein in the extract, and under the conditions used in the time course experiments we could detect gD at up to a 1:512 dilution of extract at 16 h post-adsorption (i.e. as little as 0.2% of the level of gD reached by this time could be detected). In contrast, the amount of 21K measured by Western blotting varied linearly with the amount of antigen present only at low protein concentrations. At higher concentrations, the optical density of the peak did not increase in proportion to the amount of protein present. This has the effect of underestimating the amount of 21K in the more intense peaks. In this case we could detect only 0.5 to 1% of the level of 21K reached by 16 h.

**RESULTS**

**Location of the US11 RNA cap sites**

The positions of the US11 and gD genes in the viral genome are shown in Fig. 1 (a). The US11 RNA was first identified, and its approximate 5' terminus mapped against size standards by Rixon & McGeoch (1984). We have analysed the 5' ends of US11 RNA in detail by S1 nuclease mapping. A BamHI-TagI fragment from pDG8 (Fig. 1 b) was 5' end-labelled and the appropriate strand was isolated on a strand-separating polyacrylamide gel. The probe was hybridized to RNA made at 8 h post-adsorption. S1-resistant bands were identified on an 8% polyacrylamide sequencing gel, and their sizes were determined by comparison to an A+G DNA sequence ladder made from the same probe DNA, run in the adjacent lane (Fig. 2). The 5' ends of US11 RNA map at positions 121, 123, 124 and 127 nucleotides inside Us (coordinates 12859, 12857, 12856 and 12853 of the published Us sequence; McGeoch *et al.*, 1985). These results are in close agreement with previous data (Rixon & McGeoch, 1984). The major cap site employed by US11 is at position 121, and this is indicated on the nucleotide sequence in Fig.
Fig. 1. The genome structure of HSV-1 and location of the US11 and gD genes. (a) Prototype viral genome indicating the short and long unique regions (Us and U₁), and the terminal and internal repeated regions (TR and IR). The locations and orientations of transcription of the five IE mRNAs are shown, including the spliced portions of IE mRNAs 4 and 5, which have identical 5' sequences (Preston et al., 1984). Also indicated are the US11 and gD transcript positions. (b) The 5' termini and coding sequences (open boxes) for the family of three 3' co-terminal genes located about the TRs/Us junction. Numbering refers to bp from the TRs/Us junction (coordinate 12979 of Us; McGeoch et al., 1985). Also indicated are the TRs origin of replication (hatched box; Stow & McMonagle, 1983), and the probe used for S1 mapping of US11 RNA. The probe was single-stranded, and 32P-labelled at the indicated TaqI site of pDG8 [pDG8 is a pBR322-based plasmid containing an HSV-1 insert as shown in the figure]. (c) Detail of the gD 5' terminus and coding region (open box), and the single-stranded probe used for S1 mapping of gD RNA (32P-labelled at the indicated BstNI site of pGX48). Numbering is as described by McGeoch et al. (1985), where 1 is the first base of Us at the Us/IRs junction. (d) DNA sequence of the region around the US11 5' mRNA termini (Rixon & McGeoch, 1984), indicating cap site usage of the US11 gene, as described in the text. A potential 'TATA box' at -26 relative to the major cap site (121 bp inside Us, or coordinate 12859; McGeoch et al., 1985) is underlined.
Fig. 2. US11 RNA cap sites. Lane 1, A+G sequence reaction of the 5’ end-labelled probe; lane 2, protected hybrids from S1 mapping of RNA produced 8 h after infection of BHK cells with HSV-1. The nucleotide sequence of the ‘sense’ strand (complementary to the probe) around the cap sites is shown. Interpretation of these data takes into account that the sequence track is displaced down the gel by one base compared to the S1 hybrids, due to the chemistry of the sequencing reaction.

1(d) as +1. The potential ‘TATA box’ identified by McGeoch et al. (1985) is located 26 nucleotides upstream from the major cap site (see Fig. 1).

Appearance of US11 and gD transcripts during viral infection

The time of appearance and subsequent accumulation of gD and US11 transcripts during a lytic viral infection was determined by quantitative S1 mapping of RNA using the specific, 5’ end-labelled probes described above and shown in Fig. 1(b, c). The gD promoter and cap sites have been described (Watson et al., 1983; Everett, 1983, 1984a).

US11 transcripts could be consistently detected at very low levels 2 h post-adsorption, then rose dramatically until between 6 and 10 h, and were maintained at a high level until 24 h post-adsorption (Fig. 3a and 4), despite increasing cell loss from 16 h post-adsorption onwards.

In the presence of the HSV DNA replication inhibitor, PAA, US11 transcripts were first
Fig. 3. Time course of appearance (h post-adsorption) and accumulation of US11 and gD transcripts in the presence or absence of PAA. The S1-resistant hybrids corresponding to the 5' start sites of the US11 and gD genes are indicated. (a, c) Without PAA. (b, d) PAA included before, during and after the infection at 300 μg/ml. MI, Mock-infected.

detected at 2 h post-adsorption but did not rise substantially above this level at later times (Fig. 3b and 4). Accumulation of US11 transcripts was inhibited by PAA between 50- and 100-fold (Fig. 4). The level of US11 transcripts made from mutant PAA-l-infected cells at 16 h was similar to unrestricted wild-type levels, and was unaffected by the presence of PAA (data not shown). This indicates that PAA at a concentration of 300 μg/ml did not have any indirect effects on US11 transcription. We argue below that the pattern of US11 expression is typical of a true late promoter in both its time course and sensitivity to DNA replication inhibitors.

In contrast, gD transcripts were first detected at 1 h post-adsorption, accumulated to a maximum level at between 4 and 5 h, and then gradually diminished (Fig. 3c and 4). This result
Fig. 4. Time course of appearance, accumulation and sensitivity to PAA of US11 and gD transcripts (a and b respectively) and protein products (c and d respectively) represented graphically. ○, PAA absent; ●, PAA present. Quantification of data was as described in Methods.

confirms previous findings regarding transcription of the gD gene (R. D. Everett, unpublished data). In the presence of PAA, the time course of gD expression was unchanged, but the total level of gD transcripts was reduced by five- to tenfold (Fig. 3d and 4). Thus, there is a clear difference between the regulation of transcription of gD, a delayed early gene, and US11, a true late gene.

Appearance of the 21K polypeptide and glycoprotein D during viral infection

To confirm the difference in behaviour seen between gD and US11 RNAs the appearance and accumulation of their protein products were simultaneously investigated under the above
Fig. 5. (a to d) Time course (h post-adsorption) of US11 gene product (mol. wt. 21K) during viral infection in the presence or absence of PAA. Protein products were detected by Western blotting using an oligopeptide-induced antiserum. (a) Normal time course of appearance and accumulation of US11 gene product; mol. wt. of specific bands were determined by comparison to a 35S-labelled HSV-1 standard PAGE gel (not shown; Marsden et al., 1976). (b to d) Sensitivity of 21K protein to PAA: (b) normal time course (relevant portion of gel only shown); (c) parallel time course in the presence of PAA (300 μg/ml); (d) longer exposure of (c). (e) Insensitivity of the PAAr-1 virus US11 gene product to PAA: lane 1, HSV-1 strain 17, PAA absent; lane 2, PAAr-1, PAA absent; lane 3, PAAr-1, PAA present. MI, Mock-infected.
conditions. The appearance and accumulation of 21K during viral infection was determined by Western blotting using an oligopeptide-induced antiserum specific for the US11 gene product. Fig. 5(a) shows that this serum recognized proteins of apparent mol. wt. 21K and 22K, a result consistent with the hybrid-arrest translation experiments reported by Rixon & McGeoch (1984). A number of low mol. wt. bands also present in mock-infected controls were also almost invariably seen with this antiserum. The other lower mol. wt. bands detected are possibly breakdown products of 21K. The 21K protein was first detected at 4 h post-adsorption (i.e. somewhat later than the mRNA), increased slowly to 6 h and then more rapidly, to become highly abundant by 10 to 16 h (Fig. 5). Treatment with PAA inhibited the synthesis of 21K by at least 25- to 50-fold, so that it could only be detected weakly from 6 h onwards; PAA treatment did not significantly affect the production of 21K by the PAA-resistant mutant (Fig. 5b to e). The US11 gene product induced by mutant PAAr-1 migrated more slowly on this gel system than that induced by the parental strain (Fig. 5e). We have not investigated the basis for this altered migration but it is interesting to note that in a study of several strains of HSV-1, Lonsdale et al. (1979) observed this protein to have a highly variable mobility.

The appearance and accumulation of gD during viral infection was determined by immunoprecipitation of radiolabelled gD using a gD-specific monoclonal antibody. This protein was first detected at low levels at 2 h post-adsorption (again slightly later than detection of the mRNA), was much increased by 3 h and accumulated steadily thereafter. This may appear to be in contrast to the decline in gD transcripts seen later in infection, but since only the accumulation of protein and RNA has been measured, the relative rates of their synthesis and degradation are unknown. These results could be explained if the gD RNA was relatively unstable compared to gD protein at late times after infection. Furthermore, our results cannot be compared directly with the pulse-labelling experiments of Johnson & Spear (1984), who found
gD synthesis to decrease at late times in infection, since in our experiments label was present continuously from the time of infection. In the presence of PAA, gD accumulated throughout infection but the amount detected was reduced approximately fivefold without altering the general pattern (Fig. 4b, d). Note that at 2 h post-adsorption, before detectable DNA replication (Fig. 6), the level of gD was not significantly affected by the presence of PAA.

**Accumulation of viral DNA in the presence and absence of PAA**

Total DNA was extracted from the nuclei of infected cells, and was analysed for the presence of viral DNA sequence using a nick-translated probe, pGX156, containing the entire U5 sequence. Fig. 6 shows that the amount of viral DNA detected began to increase between about 2 and 3 h after adsorption. In the presence of PAA, there was no increase in the level of virus DNA. The histogram in Fig. 6 shows that the level of background hybridization of pGX156 to DNA from mock-infected cells was similar to that seen to DNA from PAA-treated infected cells. The histogram also shows, as expected, that PAA did not inhibit DNA replication of the PAA-resistant mutant, PAAT1.

**DISCUSSION**

This paper describes the temporal regulation and sensitivity to PAA of the expression of the US11 gene of HSV-1. We have demonstrated by means of an oligopeptide-induced antiserum that a 21K polypeptide is encoded by US11, in agreement with previous sequence-derived evidence (Rixon & McGeoch, 1984). Little is known of the properties of this 21K polypeptide. However, a 21K HSV-1 protein was shown to bind to DNA (Bayliss et al., 1975) and 21K/22K proteins which are synthesized only late in infection were found to interact specifically with the 'a' sequence of HSV-1 (Dalziel & Marsden, 1984). We are currently investigating whether it is these polypeptides that are encoded by US11.

The results presented here confirm the approximate location of the 5' terminus of US11 RNA described by Rixon & McGeoch (1984), and detail the actual cap sites used. We have used reliable, specific and very sensitive probes to investigate the expression and regulation of the US11 RNA and protein products and have compared the expression of US11 with a well characterized delayed early gene, gD. The differences in the pattern of RNA and protein expression during both a productive infection and in the presence of a DNA replication inhibitor suggest that US11 is regulated as a later class of gene than gD.

'True late' or γ2 genes have been occasionally defined as those which have a stringent dependence on viral DNA synthesis for their expression, unlike the earlier β or γ1 classes whose expression is reduced, but not abolished, in the absence of DNA synthesis (Silver & Roizman, 1985). Although the level of DNA replication inhibition in experiments using either mutant virus stocks or chemical inhibitors has generally been estimated at more than 95% (Swanstrom & Wagner, 1974; Powell et al., 1975; Holland et al., 1980; Conley et al., 1981; Pedersen et al., 1981) it is not possible to conclude that absolutely no replication had taken place. Similarly, given the sensitivity of the techniques that have sometimes been used to estimate true late gene expression, it is not possible to state unequivocally that no product was present under the conditions used for DNA replication inhibition. This is so particularly where the minimum levels of detection of late gene products have not been defined (Swanstrom & Wagner, 1974; Jones & Roizman, 1979; Holland et al., 1980; Anderson et al., 1981; Conley et al., 1981; Hall et al., 1982; Silver & Roizman, 1985). In our study, specific and very sensitive probes have been used to monitor the expression of US11 products during normal infection and in the presence of PAA, an inhibitor of HSV DNA replication. It was shown that we can detect as little as 1% of the maximum amount of gene products observed and that US11 behaves as a true late gene in its time course of appearance. Having found that the appearance of US11 is extremely sensitive to inhibition of DNA synthesis we conclude that in this respect it is also directly comparable to previously defined true late genes. However, the use of sensitive techniques brings into question the absolute requirement for DNA replication for late gene expression. In view of the difficulties of measuring very low levels of DNA replication and late gene products, and in the absence of a clear understanding of the mechanism of control of late gene expression, we argue that late genes...
cannot be defined using absolute statements. An operational definition which is consistent with our data and other studies (Powell et al., 1975; Honess & Watson, 1977; Pedersen et al., 1981; Godowski & Knipe, 1985) is that true late genes are those whose expression is most severely reduced, compared to all other groups of genes, under conditions of severely inhibited DNA replication. We have deliberately not put a number on the term 'most severely reduced' but as a practical guide, a figure of 95% inhibition could be used.

Although true late genes can be operationally defined in this way, the available evidence does not allow one to reach any conclusion concerning the mechanistic relationship between DNA replication and true late gene expression. It is possible that late genes are transcribed early in infection but that, in comparison to early promoters, the true late promoter is relatively weak (i.e. also weaker than delayed early promoters) and requires a high copy number achieved through DNA replication for abundant expression. This has been suggested for the late promoter of polyoma virus and simian virus 40 (SV40) (Jat et al., 1982). Recent reports have shown that whilst DNA replication is a major factor in achieving abundant SV40 late gene expression, maximal promoter activity requires activation in trans by large T antigen (Keller & Alwine, 1984; Hartzell et al., 1984). These experiments were performed using the SV40 late promoter on plasmid constructs containing either a functional or non-functional origin of replication, which eliminates the uncertainties of inhibitor efficiency. Similar approaches are needed to determine the absolute role of DNA replication in the activation of HSV late genes.

Thus, to investigate the transcriptional switching leading to late gene expression it has been decided to study a plasmid-borne late promoter. By linking the US11 promoter to a non-viral gene we are currently studying the effects of various manipulations (including the addition or deletion of an HSV-1 origin of replication) and the requirement of trans-acting factors on the activity of the promoter by detecting unique transcripts in transfected and superinfected cells.

We are grateful to Dr Dairena Gaffney for pDG8, Dr Frazer Rixon for pGX48, Dr Anne Cross for anti-gD monoclonal antibody 1892, and Mrs Mary Murphy for seed stocks of the mutant virus, PAA*-1. For critically reading the manuscript we are most grateful to Professor J. H. Subak-Sharpe. P.A.J. was supported by a SERC studentship, C.M. by a University of Glasgow postgraduate scholarship, and R.G.D. by a MRC studentship.

REFERENCES


RIXON, F. J. & MCGEOCH, D. J. (1984). A 3' co-terminal family of mRNAs from the herpes simplex virus type 1 short region: two overlapping reading frames encode unrelated polypeptides one of which has a highly reiterated amino acid sequence. Nucleic Acids Research 12, 2473–2487.


(Received 31 October 1985)