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Trans activation of transcription by herpes virus products: requirement for two HSV-1 immediate-early polypeptides for maximum activity

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The transcriptional programme of the herpes viruses is organised into three principal phases. The immediate-early (IE) genes are the first to be transcribed, by the pre-existing host RNA polymerase II, and their promoters are strongly stimulated by a polypeptide component of the virus particle. The E and L gene promoters become active only after the appearance of IE gene products. Genetic and biochemical evidence has shown that the HSV-1 IE polypeptide Vmw175 (ICP 4) is essential for the *trans* activation of HSV early promoters, but the role of none of the other four IE gene products was known. This paper describes functional tests that show, by co-transfection of recombinant plasmids into HeLa cells, that (i) Vmw175 alone can activate an HSV-1 E gene promoter, (ii) the four other HSV-1 IE gene products by themselves are unable to activate transcription, (iii) the combination of Vmw175 plus the product of IE gene 1, Vmw110 (ICP 0), is a much better activator than Vmw175 alone, (iv) cloned IE gene products of human cytomegalovirus (CMV), varicella-zoseter virus (VZV) and pseudorabies virus (PRV) can also activate transcription from an HSV-1 early promoter, and (v) this activation also occurs with cellular promoters.

Key words: herpes virus/immediate-early/*trans* activation/transcription

Introduction

Herpes virus virions are large enveloped icosahedral structures containing large linear double-stranded DNA genomes (for a review, see Tooze, 1981). In the case of herpes simplex virus-1 (HSV-1), there are >50 identified virally encoded polypeptides found in the productively infected cell (Honess and Roizman, 1974; Marsden *et al.*, 1976). As many as 230 virus-induced polypeptides have been detected by two-dimensional gel electrophoresis (Haar and Marsden, 1981). These polypeptides are translated from viral mRNA molecules which have been classified into three temporal groups (immediate-early, early and late or α, β and γ) which are regulated in a cascade fashion (Swanstrom and Wagner, 1974; Clements *et al.*, 1977; Jones and Roizman, 1979). The IE RNA species are transcribed by the host cell RNA polymerase II (Costanzo *et al.*, 1977). If protein synthesis is blocked from the onset of infection, or if the mutant tsK (an allele of IE gene 3 coding for a thermosensitive Vmw175) is used at the non-permissive temperature, infection fails to proceed because transcription of the early and late genes is not initiated (Preston, 1979a; Watson and Clements, 1980). Thus the activation of E gene promoters by one or more IE gene products is fundamental to productive viral infection.

Recently, a short-term transfection procedure has been used to investigate the nature of the DNA sequences within the early HSV-1 glycoprotein D (gD) promoter which are necessary for activation during virus infection (Everett, 1983, 1984). These studies indicate that all the sequences necessary for full response to viral activation lay between nucleotides −83 and +17 relative to the gD cap sites. The DNA sequences in this region which were required for *trans* activation by viral products could not be distinguished from those involved in *cis* activation in the absence of viral products. These studies have been extended to show that a large variety of herpes viruses can activate both viral and cellular promoters in this assay (Everett and Dunlop, 1984). Similar results have been obtained using adenovirus-infected transfected cells (Green *et al.*, 1983) and cells transfected with plasmids containing either the adenovirus E1A or the pseudorabies virus IE gene (Imperiale *et al.*, 1983; Green *et al.*, 1983; Svensson and Akusjarvi, 1984).

This report describes experiments which were designed to investigate the role of various herpes virus IE gene products in the *trans* activation of viral early promoters. The approach used was to simultaneously co-transfect into HeLa cells plasmids containing various viral IE genes with other plasmids containing either the HSV-1 gD promoter linked to the rabbit β-globin gene, or the complete rabbit β-globin gene with its own promoter. The RNA transcribed from the activated gD or β-globin promoter was quantitated by S1 mapping analysis of total cytoplasmic RNA prepared 52 h after transfection.

The results show that although the HSV-1 IE gene 3 product, Vmw175, can activate transcription on its own, its effect is substantially increased when plasmids expressing the product of IE gene 1, Vmw110, is also present. This is the first report of the possible function of Vmw110. The other IE gene products of HSV-1 appeared not to have a detectable effect in this assay. The strong transcriptional activation mediated by the pseudorabies virus (PRV) IE gene product observed previously (Green *et al.*, 1983; Imperiale *et al.*, 1983) is confirmed. In addition, the IE gene products of varicella-zoster virus (VZV) and human cytomegalovirus (CMV) were also active in this assay. These data imply that the mechanism of herpes virus activation of transcription in *trans* involves interactions between cellular RNA polymerase II (and perhaps other factors), one or more viral products and DNA which is not apparently identified by viral specific sequences.

Results

Transcriptional activation of the gD promoter after co-transfection with plasmids containing HSV-1 IE genes

It has been shown in short-term transfection experiments (Everett, 1983, 1984; Everett and Dunlop, 1984) and in biochemically transformed cell lines (Leiden *et al.*, 1976; Sandri-Goldin *et al.*, 1983; Smiley *et al.*, 1983), that both plasmid-encoded and integrated viral promoters can be activated during HSV-1 infection. Earlier studies using protein syn-

thesis inhibitors during HSV-1 infection had established that IE gene products were required for early gene expression (Clements *et al.*, 1977) and that the product of IE gene 3, Vmw175, was essential (Preston, 1979a; Watson and Clements, 1980). However, it was not clear whether any of the other IE gene products were involved in transcriptional regulation because no virus mutants carrying lesions in these genes were available. A virus strain carrying a truncated version of IE gene 4 product, Vmw68 (ICP 22), has been described (Post and Roizman, 1981). This strain did not appear to be defective in Vero cells, but it appears that an intact Vmw68 polypeptide is required for virus growth in some cell lines (I.Halliburton, personal communication).

The availability of recombinant plasmids containing one or more IE genes allows the functions of the IE gene products to be tested in short-term transfection assays. In addition, possible interaction or cooperation between the IE gene products can be studied by co-transfection of more than one of these plasmids at the same time. The structure of the HSV-1 genome, the locations of the five IE genes and the plasmids used in this study which carry these genes, are shown in Figure 1. In some cases the plasmids contain not only the IE gene of interest, but also other regions of HSV DNA. Plasmid pGX156 contains *Eco*RI fragment h which encompasses the whole of the U_S region and includes the coding sequences of IE genes 4 and 5 (Vmw68 and Vmw12). The identical promoter sequences of these two IE genes are wholly contained within the section of the short repeat region in *Eco*RI h (Preston *et al.*, 1984a). The U_S region, which has been fully sequenced, directs the synthesis of 10 other E or L transcripts, and each one contains an open reading frame coding for both identified and unidentified polypeptides (McGeoch *et al.*, 1984). Plasmid pGXS19 contains a 15.5-kb fragment of the U_L and IR_L regions spanning *Bam*HI fragment b and parts of *Bam* l (to the left of *Bam* b) and *Bam* k (Figure 1). This plasmid contains IE genes 1 and 2, which code for Vmw110 and Vmw63 (ICP 27), respectively. The region of *Bam* b between IE genes 1 and 2 is transcribed into three late RNAs detectable on Northern blots (F.J.Rixon, personal communication); the 3-kb portion of *Bam* l contained in both pGXS19 and pGXS12 (Figure 1) is of unknown function. Plasmid pJR3 contains IE gene 1 and probably little other coding DNA (Figure 1). Plasmid pGX58 contains *Xho*I fragment c, which includes IE gene 3 (coding for Vmw175) and 865 bp at the 5′ end of the 1260-bp coding region of IE gene 4. Finally, pGX33 (*Bam*HI fragment n) contains the whole of IE gene 4 and a further 2.3 kb of U_S which includes one other complete transcription unit. These plasmids enable each of the IE gene products (with the exception of Vmw12) to be studied in the absence of any of the other IE genes and also in combination with each other. The promoters of all of the IE genes are known to be constitutively active in tissue culture cells (Mackem and Roizman, 1982a, 1982b; Cordingly *et al.*, 1983; Preston *et al.*, 1984a).

The test plasmids used, which contain the HSV-1 gD promoter (pRED4) or the rabbit β-globin promoter (pβdel) have been described (Everett, 1983; Everett and Dunlop, 1984) and are shown in Figure 2. HeLa cells were transfected with pRED4 and pβ(244+)β (de Villiers and Schaffner, 1981), and one or more plasmids containing single or combinations of IE genes. Plasmid pβ(244+)β contains the rabbit β-globin gene linked to the polyoma virus enhancer and therefore acts as a control for each transfection since it constitutively expresses correctly initiated β-globin RNA which results in bands at 136

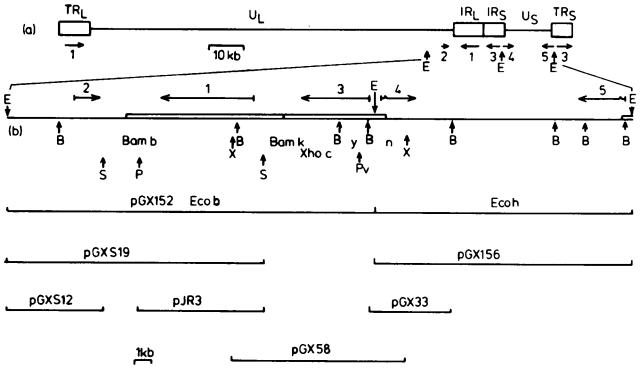

Fig. 1. The structure of HSV-1 DNA, the positions of the IE genes and the cloned fragments used. **(a)** The HSV-1 genome in the prototype arrangement with the positions and direction of transcription of IE genes 1–5. IR = internal repeat, TR = terminal repeat, U = unique region, L = large, S = small. The *Eco*RI sites bounding *Eco*RI fragments b and h are shown (E). **(b)** Partial restriction map and accurate locations of IE genes 1–5 in *Eco*RI fragments b and h. B = *Bam*HI, X = *Xho*I, S = *Sst*I, P = *Pst*I, Pv = *Pvu*II. *Bam*HI fragments b, k, y and n, and *Xho*I fragment c are marked. The positions of the 5′ and 3′ ends of the IE RNAs are taken from Mackem and Roizman (1982a, 1982b) and Whitton *et al.* (1983) (IE-1 and IE-2), Rixon *et al.* (1982) (IE-3) and Rixon and Clements (1982) (IE-4 and IE-5). The HSV-1 DNA fragments cloned in pGX152, pGX156, pGXS19, pGXS12, pJR3, pGX33 and pGX48 are shown below to the same scale.

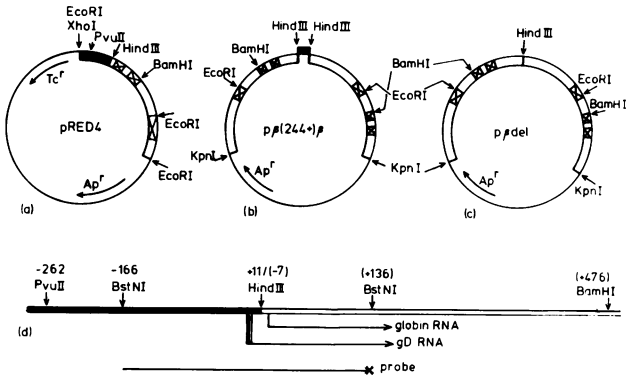

Fig. 2. Structures of the test plasmids used containing the HSV-1 gD and rabbit β-globin promoters. **(a)** pRED 4. A 409-bp *Xho*I-*Hind*III fragment containing the gD promoter from positions −392 to +11 (filled arc) is upstream of the rabbit β-globin gene (open arc) with the exons shown as crossed boxes. The vector (single line) is a *Bam*HI^r Tet^r derivative of pBR322. **(b)** pβ(244+)β. Single copies of the rabbit β-globin gene are cloned into pBR322, with the 244-bp *PvuII-BclI* fragment of polyoma virus containing its enhancer region present between two *Hind*III sites. **(c)** pβdel is a derivative of pβ(244+)β; the polyoma enhancer has been deleted. **(d)** The junction between gD and globin sequences in pRED 4. The cap sites of gD and globin RNA are shown. The globin starts (shown here for convenience) are derived from the normal β-globin promoter in pβ(244+)β and pβdel and are not used in pRED4 (Everett, 1983). The probe for S1 mapping was a single-stranded fragment 5′ end-labelled at the *Bst*NI site in the β-globin region at position +136 and was derived from the *Pvu*II-*Bam*HI fragment spanning the junction region. The same probe detected both the hybrid gD/β-globin transcripts from pRED4 and the shorter authentic β-globin RNA. This probe did not hybridise to transcripts from untransfected cells (results not shown).

nucleotides on the gel (Figures 3 and 5). Two days after transfection, total cytoplasmic RNA was prepared and analysed by S1 nuclease digestion of hybrids formed between the RNA and a single-stranded end-labelled probe which detects transcripts initiated at both the gD and the β-globin promoters (Figure 2). The results are given in Figure 3. Plasmid pGX58,

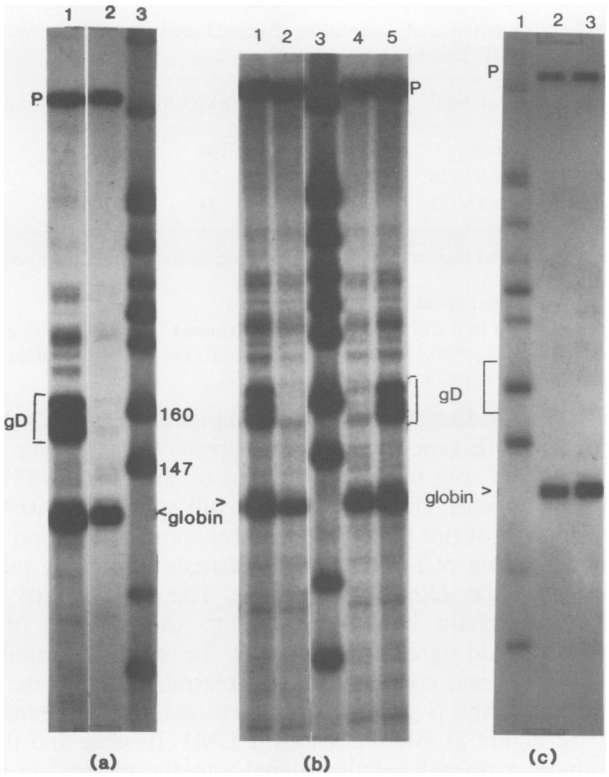

Fig. 3. Activation of the gD promoter by HSV-1 IE gene products. The mol. wt. markers are *Hpa*II-cut pBR322. The bands in all experimental tracks at the top of the gel (marked P) are at the position of full length probe. In all experiments pRED4 and pβ(244+)β were transfected together with other plasmids containing one or more IE genes as indicated. The characteristic triple group of gD 5′ ends, the doublet band of β-globin RNA and the marker bands at 147 and 160 nucleotides are indicated. After prolonged exposures some background material is visible in the region of gD 5′ ends, but this is only taken as gD RNA when the normal triplet pattern is visible. **(a) Track 1:** pGXS19 + pGX58 + pGX156. **Track 2:** pGX58. **Track 3:** standards. **(b) Track 1:** pGX58 + pGX156 + pJR3. **Track 2:** pGXS19 + pGX156. **Track 3:** standards. **Track 4:** pGX58. **Track 5:** pGX58 + pJR3. **(c) Track 1:** standards. **Track 2:** no activating plasmids. **Track 3:** pJR3. The band marked P is the full-length probe.

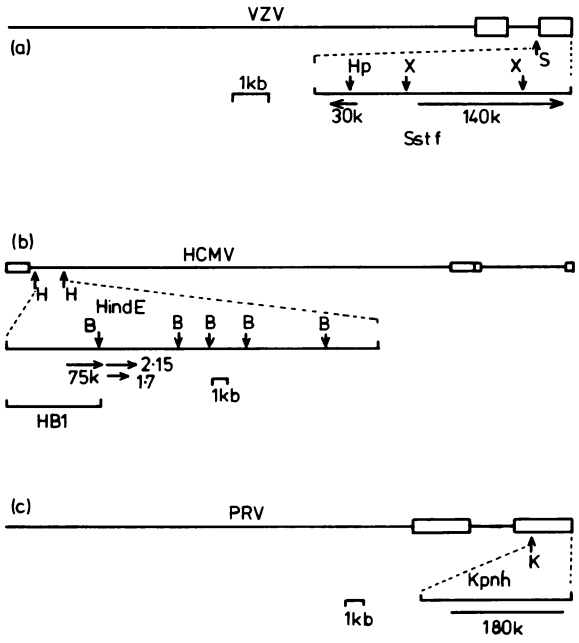

Fig. 4. The structures of VZV, CMV and PRV DNA, the positions of their IE genes and the cloned fragments used. The scale in each case refers to the clone maps. The boxed regions on the genome maps indicate terminal and internal repeated sequences. The short repeats bounding U_L in VZV (A.Davison and J.Scott, in preparation) are not shown. S = *Sst*I, Hp = *Hpa*I, X = *Xho*I, H = *Hind*III, B = *Bam*HI, K = *Kpn*I. **(a)** VZV. *Sst* fragment f (cloned in pVZVSstf) extends from the genome terminus and includes the whole or TR_S which contains the open reading frames marked. pVZVdel is deleted between the two indicated *Xho*I sites. pVZVins contains *Sst*I linker insertions into the *Hpa*I site. **(b)** HCMV strain AD169. *Hind*III fragment E is cloned in pCMVHindE, pCMVHB1 contains fragment HB1. The major CMV IE RNA which encodes the 75-K polypeptide is marked. Two less abundant IE RNAs of 2.15 kb and 1.7 kb are also indicated. Other minor transcripts originating from this and other regions of the genome can also be detected at IE times (Wilkinson *et al.*, 1984). **(c)** PRV. The 6-kb major IE transcript encodes a 180-K polypeptide and is entirely contained within *Kpn*I fragment h (cloned in pPRVKpnh). The precise location of this gene is not known (Ihara *et al.*, 1983).

which contains a complete IE gene 3 expressing Vmw175, just perceptively activated transcription from the gD promoter (Figure 3a track 2 and 3b track 4) while a combination of plasmids pGXS19 and pGX156 (containing IE genes 1, 2, 4 and 5) did not detectably activate the gD promoter (Figure 3b track 2). When all the IE genes were present (using pGX58, pGXS19 and pGX156 together) the activation of the gD promoter was increased 12- to 60-fold (Figure 3a track 1). A high level of transcription within this range also occurred with pGX58 plus pGXS19 (IE genes 1, 2 and 3) (Figure 3b track 1) and with pJR3 plus pGX58 (IE genes 1 and 3 only) (Figure 3b track 5). These results clearly show that the maximum stimulation of gD transcription was achieved when both IE genes 1 and 3, coding for Vmw110 and Vmw175, were present. Vmw110 was unable, either by itself (Figure 3c) or in combination with other IE genes (excluding IE gene 3), to activate the gD promoter (Figure 3). Similarly, plasmids pGX33 (containing Vmw68), pGX156 (containing both Vmw68 and Vmw12) and pGXS12 (containing Vmw63) failed to stimulate gD transcription when used either singly or in combination (results not shown). The addition of IE genes 2, 4 and 5 to co-transfections of IE genes 1 and 3 in several other experiments did not appear to increase gD transcription further than the level seen with IE genes 1 and 3 in combination. Finally, the effect observed with IE gene 3 alone was not increased by the inclusion of IE genes 2, 4 and 5 (data not shown). Thus the maximum effect appears to be obtained by the presence of Vmw175 and Vmw110 together. This is the first indication of the functional role of Vmw110.

Transcriptional activation of the HSV-1 gD promoter after co-transfection of plasmids containing IE genes from heterologous herpes viruses

Infection with PRV, VZV or CMV of cells transfected with plasmids containing the HSV-1 gD promoter has been shown to result in a stimulation of transcription from this promoter (Everett and Dunlop, 1984). The viral gene products responsible for this effect were investigated in HeLa cells by co-transfection of plasmids containing the IE genes of PRV, VZV or CMV with pRED4 and pβ(244+)β, followed by analysis of total cytoplasmic RNA exactly as in the experiments using HSV-1 IE genes. Approximate genetic maps of the cloned DNA fragments used and their locations in the viral genomes are shown in Figure 4. The results (Figure 5) demonstrate that plasmids pPRVKpnh, pVZVSstf and pCMVHindE stimulate transcription from the gD promoter. This effect of the PRV IE gene has been reported before (Green *et al.*, 1983; Imperiale *et al.*, 1983). The *Kpn* h region

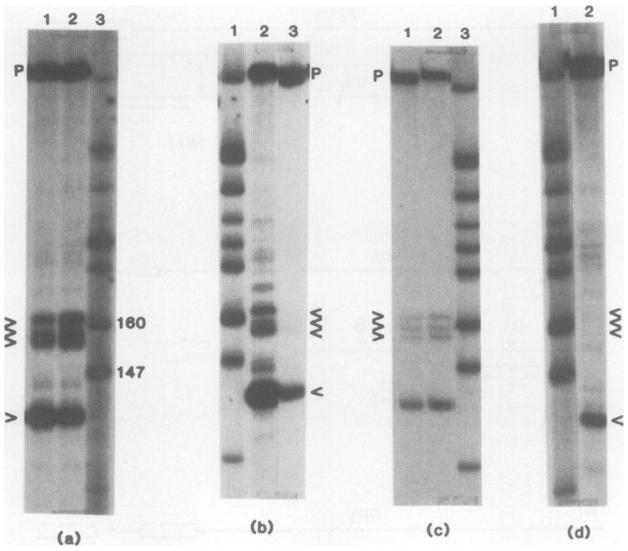

Fig. 5. Activation of the gD promoter by heterologous herpes virus IE gene products. Mol. wt. markers are shown in **(a) track 3** (with the 160 and 147 nucleotide bands marked), **(b) track 1**, **(c) track 3** and **(d) track 1**. P marks the position of the full-length probe. **(a)** pRED4 and pβ(244+)β transfected with pVZVSstf **(track 1)**, or pPRVKpnh **(track 2)**. **(b)** pRED4 and pβ(244+)β transfected with pCMVHindE **(track 2)** or pCMVHB1 **(track 3)**. **(c)** pRED4 and pβdel transfected with pVZVins **(track 1)** or pVZVSstf **(track 2)**. **(d)** pRED4 and pβ(244+)β transfected with pVZVdel **(track 2)**. The triple and single arrows indicate the positions of gD and β-globin 5′ ends, respectively.

of PRV is transcribed to produce a major IE RNA encoding a polypeptide of 180 K, but it is not known if any other viral products are derived from this region (Ihara *et al.*, 1983).

On the basis of the complete DNA sequence, the *Sst* f region of VZV contains two major open reading frames of 140 K and 30 K which show homology to Vmw175 and Vmw68 of HSV-1, respectively (A.Davison and J.Scott, in preparation). The homology suggests that these genes are equivalent to the IE genes of HSV-1, although their time of appearance during VZV infection is unknown. The roles of these two sequence-predicted polypeptides were analysed by constructing derivatives of pVZVSstf which were deleted in the large open reading frame (pVZVdel) or contained a frame-shift mutation near the N-terminal end of the smaller open reading frame (pVZVins) (Figure 4a). Plasmid pVZVins was as equally effective as pVZVSstf, but pVZVdel was unable to activate the gD promoter (Figure 5c track 1 and Figure 5d track 2). These results suggest that the predicted product of the large open reading frame in pVZVSstf is probably the only active polypeptide involved in this transcriptional activation.

The *Hind* E region of CMV strain AD169 contains several transcription units, some of which have been classified as immediate-early (McDonough and Spector, 1983; Stinski *et al.*, 1983; Wilkinson *et al.*, 1984). The major IE gene transcript encodes a 75 K polypeptide and is located almost entirely (except for the polyadenylation signal) within a *Hind*III-*Bam*HI fragment (Wilkinson *et al.*, 1984; A.Akrigg, G.W.G.Wilkinson and J.D.Oram, personal communication); plasmid pCMVHB1 contains this fragment cloned into pAT153 (Figure 4). In contrast to the result obtained with pCMVHindE (Figure 5b track 2), the amount of transcriptional activation from the gD promoter was scarcely detectable when pCMVHB1 was used in co-transfection experiments (Figure 5b track 3, but see also Figure 6b track 3). The major CMV IE gene product thus appears to have some activity by itself, but may be analogous to Vmv175 of HSV-1 and require other viral products for full activity. However, confirmation of this possibility requires complementation experiments using pCMVHB1 with plasmids containing other regions of the *Hind* E fragment. The low activity of pCMVHB1 could have been due to the absence of a polyadenylation signal after the gene. To test this possibility pCMVHBA1 was constructed. This plasmid contains the 3′ end of the rabbit β-globin gene (downstream of the *Bam*HI site, see Figure 2) inserted after the CMV IE gene and thus introduces a polyadenylation signal into the plasmid in the correct orientation. Plasmid pCMVHBA1 was no more active than pCMVHB1 in activating transcription from the gD promoter (results not shown), so it appears that the low activity was not due to the lack of a polyadenylation signal. The ability of the CMV plasmids to activate transcription from the gD promoter in HeLa cells contrasts with the inability of CMV virus to stimulate the same promoter during infection of the (non-permissive) HeLa cells (Everett and Dunlop, 1984). The positive effect of pCMVHindE suggests that the block to CMV infection in HeLa cells is at a very early stage in infection, rather than an inability of the IE gene products to function in these cells.

Table I. Quantitation of the activation of the gD promoter in *trans* by the VZV and PRV IE gene products

Plasmid	Mutation[a]	HSV infection[b]	pVZVSstf[c]	pPRVKpnh[c]
pRED2	None	100	100	100
pERD6.123	del-67/-56	14	28	29
pRED213	ins-22/-23	23	24	12

[a]The plasmids and their mutations are described in the text and in Everett (1984).
[b]Data from Everett (1984).
[c]The ratio of gD to β-globin 5′ ends was determined for pRED2 with each activator (Everett, 1984). The ratios obtained with the promoter mutants are expressed as a percentage of that of pRED2.

Mutations in the gD promoter decrease by similar extents activation by HSV-1 and cloned IE genes from PRV and VZV

A series of mutations in the gD promoter have been analysed for their effects on transcriptional activation in *trans* during HSV-1 infection and in *cis* with the SV40 enhancer (Everett, 1984). This study indicated that there were no detectable sequences within the essential promoter region which were uniquely involved in HSV-1 specific *trans* activation. The high level of activation of the gD promoter during co-transfection with the PRV and VZV IE gene plasmids allows a comparative study of the effects of the same promoter mutations on activation by these heterologous IE gene products. The plasmid mutants chosen for study were pERD6.123, which has a small deletion in an essential upstream portion of the gD promoter, and pRED213, which contains a single *Xho*I linker inserted in the TATA box (Everett, 1984). These plasmids were transfected into HeLa cells together with pβ(244+)β (to act as an internal control) and either pVZVSstf or pPRVKpnh. Parallel experiments were performed with pRED2 for comparison with the 'wild-type' promoter. The results are summarised in Table I. It seems that the same promoter mutants affect the efficiency of *trans* activation by these different viral products to approximately similar ex-

tents. This supports the hypothesis that *trans* activation during herpes virus infection does not involve recognition by IE gene products of virus specific sequences within the promoter.

Cloned IE gene products of HSV-1, PRV, VZV and CMV can activate transcription from the rabbit β-globin promoter

Our earlier finding that a plasmid-borne rabbit β-globin promoter is transcriptionally activated during infection by a wide variety of herpes viruses illustrated that viral *trans* activation is not restricted to viral promoters (Everett and Dunlop, 1984). To extend this observation, co-transfection experiments similar to those described above were performed using pβdel, which contains the complete rabbit β-globin promoter without a *cis* acting enhancer (Figure 2) and is thus dependent on *trans* activation for transcription (Everett and Dunlop, 1984). The results demonstrate that all the IE gene products which activated (either singly or in combination) the gD promoter also efficiently activated the rabbit β-globin promoter (Figure 6).

Densitometric analysis of autoradiographs from several independent experiments gave the following average ratios of intensity of the gD/β-globin 5′ ends: VSV 0.61 (six determinations, range 0.55−0.74), PRV 3.1 (5, 2.12−4.03), CMV 0.27 (3, 0.12−0.45), and HSV (IE-1 and 3) 1.25 (5, 0.38−2.25). In general, the consistency of the ratios for each system suggests a differential specificity of the various viral activators for the gD and β-globin promoters. This is intriguing because no sequence specifically and solely responsible for activation of the gD promoter during HSV-1 infection has been detected (Everett, 1984). One possible reason for the apparent promoter choice observed here could be that the viral IE gene products interact with sequences involved in general promoter activity, such as the TATA box. Because such sequences are not identical in all promoters, a given IE gene product could activate different promoters to different extents. Differential activation is currently being studied in more detail using a variety of promoters and cell types.

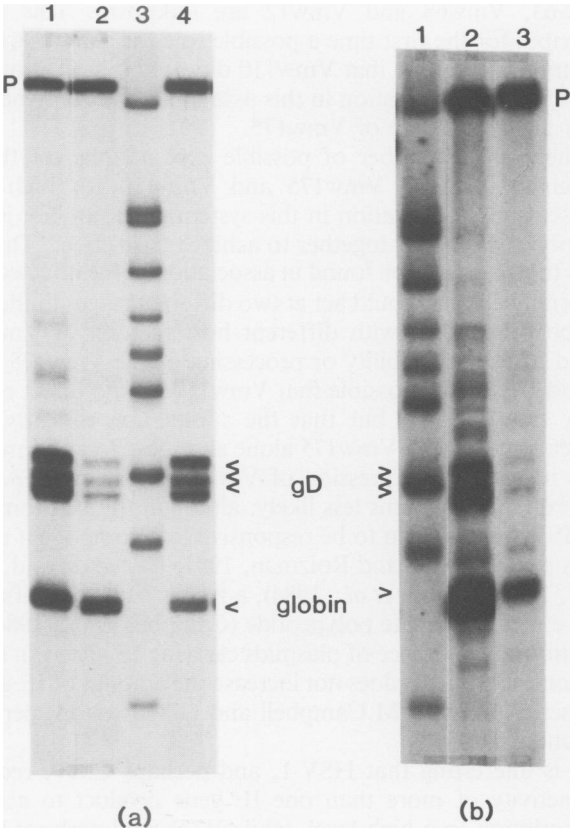

Fig. 6. Activation of the rabbit β-globin promoter by herpes virus IE gene products. Mol. wt. markers are in **(a) track 3** and **(b) track 1**. P indicates the full-length probe position. The gD and β-globin 5′ ends are marked with triple and single arrows, respectively. **(a)** pRED4 and pβdel were transfected with pGX58 and pJR3 **(track 1)**, pVZVSstf **(track 2)** or pPRVKpnh **(track 3)**. **(b)** pRED4 and pβdel were transfected with pCMVHindE **(track 2)** or pCMVHB1 **(track 3)**.

Discussion

The three principal observations described here are, first, the requirement for both Vmw175 and Vmw110 for maximum activation of the gD promoter linked to the rabbit β-globin gene, second, the ability of plasmids containing heterologous viral IE genes to activate the HSV-1 gD and rabbit β-globin promoters and third, the differential promoter choice exerted by the different viral activators. The products of HSV-1 IE-genes 1 and 3 together are a striking 12- to 60-fold more effective than IE gene 3 alone. This conclusion arises from short-term co-transfection studies using a variety of recombinant plasmids. The basic features of this experimental system are (i) the gD promoter of the hybrid gD/globin gene in pRED4 is silent unless activated by *trans* (Everett, 1983; Figure 3c), (ii) activation of the gD promoter in the plasmid during HSV-1 infection follows the same time-course and uses the same cap sites as the authentic gD promoter in the viral genome (Everett, 1983), (iii) activation of both viral and plasmid-borne gD promoters depends on expression of IE functions of HSV-1 with Vmw175 recognised as indispensable (Everett, 1983). Therefore the behaviour of the transfected gD promoter in this assay appears to be indistinguishable from its viral counterpart during normal viral infection; this implies that the observations on IE gene product requirements reported in this paper are likely to apply to normal viral infection.

At first sight it appears that the analysis of some of the results of the co-transfection experiments could be complicated by the still incomplete characterisation of the transcripts and protein products derived from some of the cloned DNA fragments. However, plasmids pJR3 and pGX58 contain well characterised regions of the viral genome which encode only IE genes 1 and 3, respectively, and no other complete transcription units. Therefore this study clearly shows that the combination of the products of IE genes 1 and 3 is sufficient to bring about a high level of transcriptional activation. The products of IE genes 2, 4 and 5 did not appear to have any additional effect in these experiments.

Little is known about the properties of the HSV-1 IE polypeptides. All, except Vmw12, are phosphorylated DNA-binding proteins which are found in the nucleus (Pereira *et al.*, 1977; Hay and Hay, 1980; Marsden *et al.*, 1982). Vmw12 is found mostly in the cytoplasm (Preston, 1979b). Vmw175 is a poly-ADP(ribosyl)ated, and the extent of this modification appears to have some correlation with the functional state of the polypeptide (Preston and Notorianni, 1983). The DNA binding property of Vmw175 is lost after extensive purification and it is thought to bind DNA only in the presence of as yet unknown host factors (Freeman and Powell, 1982). This suggests that Vmw175 might be involved in the modification of host transcription factor activity rather than in direct recognition of viral DNA. The functions of

Vmw63, Vmw68 and Vmw12 are unknown. This paper describes for the first time a possible role for Vmw110, but it is important to note that Vmw110 did not by itself show any transcriptional activation in this assay; its effect only became clear in the presence of Vmw175.

There are a number of possible explanations for the requirement of both Vmw175 and Vmw110 for high level transcriptional activation in this system. The simplest is that the polypeptides act together to achieve their effect. This implies that they may be found in association in the infected cell. Alternatively they could act at two different stages in the activation process, or with different host factors, or Vmw110 could affect the stability or processing of the Vmw175 polypeptide. It is also possible that Vmw110 has no direct role in early transcription, but that the stimulation of early promoters is caused by Vmw175 alone and the effect of Vmw110 is to increase the expression of Vmw175 from pGX58. This last explanation seems less likely: although the IE promoters of HSV-1 are known to be responsive to a component of the virus particle (Post and Roizman, 1981; Batterson and Roizman, 1983; Preston *et al.*, 1984), a recent paper identifies the active factor as a late polypeptide (Campbell *et al.*, 1984). In addition, the presence of plasmids carrying IE gene 1 in transfection experiments does not increase the amount of IE gene 3 transcription (M.E.M.Campbell and C.M.Preston, personal communication).

It is interesting that HSV-1, and perhaps CMV, requires the activity of more than one IE gene product to activate transcription to a high level, while VZV, and perhaps PRV, apparently need only one. It is possible that the mechanism(s) induced in these two latter cases are different or that the single gene product is able to serve the functions of both the required HSV IE gene products. DNA sequence analysis of Vmw175 and the VZV 140-K polypeptide has revealed regions of homology which include the position of the base change in tsK (A.Davison and J.Scott, in preparation). It is not known if there exists any sequence homology between Vmw110 and any of the heterologous viral products required for transcriptional activation. Surprisingly, there does not appear to be any significant homology between the sequence of the major IE gene product of CMV Towne strain (Stenberg *et al.*, 1984) and Vmw175 (D.McGeoch, personal communication), but it is not known whether any homology exists between other CMV IE genes and Vmw175, or between the major CMV IE polypeptide and Vmw110.

There may well be a common mechanism of activation of transcription mediated by the various viral IE gene products because the activities of these polypeptides are similar in functional assays. However, deeper insight into the method by which promoter stimulation in *trans* is achieved requires the development of *in vitro* systems that reproduce the *in vivo* effects of the viral IE gene products.

Materials and methods

Plasmids and cells

The following plasmids have been described previously: pRED2, pRED4 (Everett, 1983), pERD6.123, pRED213 (Everett, 1984), pβdel (Everett and Dunlop, 1984), pβ(244+)β (de Villiers and Schaffner, 1981), pVZVSstf (Davison and Scott, 1983) and pPRVKpnh (Davison and Wilkie, 1983). The HSV DNA restriction fragments cloned in pGX58 (*Xho*I c), pGX152 (*Eco*RI b), pGX156 (*Eco*RI h) and pGX33 (*Bam*HI n) are shown in Figure 1. The tissue culture cell lines used in these experiments were HeLa cells grown in Dulbecco's Modified Eagle's Medium (Flow Laboratories) supplemented with 2.5% foetal calf serum and 2.5% calf serum.

Construction of pGXS series plasmids

The large *Eco*RI-*Pvu*II fragment of pGX152 (Figure 1) was cloned between the *Eco*RI and *Pvu*II sites of pBR322 to give pGXD18. pGXD18 was cut with *Pvu*II, then partially with *Sst*I, the 3′ protruding extremities were removed with *E. coli* DNA polymerase I Klenow fragment and finally the DNA was ligated in the presence of *Sst*I linkers. pGXS12 and pGXS19 were isolated after screening of small plasmid preparations. Their structures are given in Figure 1.

Calcium phosphate transfection, isolation of total cytoplasmic RNA and analysis of RNA by S1 nuclease mapping

These methods were exactly as described (Everett, 1983, 1984). In all transfections 10 μg of each plasmid was used. This led to a difference in total DNA concentration between some experiments, but did not of itself affect the results (data not shown). 20 μg of RNA was hybridised overnight to the relevant strand-separated 5′ end-labelled probe (Figure 2). The hybrids were treated with S1 nuclease and the protected DNA fragments were analysed on 8% acrylamide gels containing 7 M urea. The gels were analysed by autoradiography, and densitometry where necessary.

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References

Batterson,W. and Roizman,B. (1983) *J. Virol.*, **46**, 371-377.
Campbell,M.E.M., Palfreyman,J. and Preston,C.M. (1984) *J. Mol. Biol.*, in press.
Clements,J.B., Watson.R.J. and Wilkie,N.M. (1977) *Cell*, **12**, 275-285.
Cordingly,M.G., Campbell,M.E.M. and Preston,C.M. (1983) *Nucleic Acids Res.*, **11**, 2347-2365.
Costanzo,F., Campadelli-Fiume,G., Foa-Tomasi,L. and Cassai,E. (1977) *J. Virol.*, **21**, 996-1001.
Davison,A.J. and Scott,J.E. (1983) *J. Gen. Virol.*, **64**, 1811-1814.
Davison,A.J. and Wilkie,N.M. (1983) *J. Gen. Virol.*, **64**, 1927-1942.
de Villiers,J. and Schaffner,W. (1981) *Nucleic Acids Res.*, **9**, 6251-6264.
Everett,R.D. (1983) *Nucleic Acids Res.*, **11**, 6647-6666.
Everett,R.D. (1984) *Nucleic Acids Res.*, **12**, 3037-3056.
Everett,R.D. and Dunlop,M. (1984) *Nucleic Acids Res.*, **12**, 5969-5978.
Freeman,M.J. and Powell,K.L. (1982) *J. Virol.*, **44**, 1084-1087.
Green,M.R., Treisman,R. and Maniatis,T. (1983) *Cell*, **35**, 137-148.
Haarr,L. and Marsden,H.M. (1981) *J. Gen. Virol.*, **52**, 77-92.
Hay,R.T. and Hay,J. (1980) *Virology*, **104**, 230-234.
Honess,R.W. and Roizman,B. (1974) *J. Virol.*, **14**, 8-19.
Ihara,S., Feldman,L., Watanabe,S. and Ben-Porat,T. (1983) *Virology*, **131**, 437-454.
Imperiale,M.J., Feldman,L.T. and Nevins,J.R. (1983) *Cell*, **35**, 127-136.
Jones,P.C. and Roizman,B. (1979) *J. Virol.*, **31**, 299-314.
Leiden,J.M., Buttyan,R. and Spear,P.G. (1976) *J. Virol.*, **20**, 413-424.
Mackem,S. and Roizman,B. (1982a) *J. Virol.*, **43**, 1015-1023.
Mackem,S. and Roizman,B. (1982b) *J. Virol.*, **44**, 939-949.
Marsden,H.S., Crombie,I.K. and Subak-Sharpe,J.S. (1976) *J. Gen. Virol.*, **31**, 347-373.
Marsden,H.S., Lang,J., Davison,A.J., Hope,R.G. and Macdonald,D.M. (1982) *J. Gen. Virol.*, **62** 17-27.
McDonough,S.H. and Spector,D.H. (1983) *Virology*, **125**, 31-46.
McGeoch,D.J., Dolan,A., Donald,S. and Rixon,F.J. (1984 *J. Mol. Biol.*, in press.
Pereira,L., Wolff,M.H., Fenwick,M. and Roizman,B. (1977) *Virology*, **77**, 733-749.
Post,L.E. and Roizman,B. (1981) *Cell*, **25**, 227-232.
Post,L.E., Mackem,S. and Roizman,B. (1981) *Cell*, **24**, 555-565.
Preston,C.M. (1979a) *J. Virol.*, **29**, 275-284.
Preston,C.M. (1979b) *J. Virol.*, **32**, 357-369.
Preston,C.M. and Notarianni,E.L. (1983) *Virology*, **131**, 492-501.
Preston,C.M., Cordingley,M.G. and Stow,N.D. (1984) *J. Virol.*, **50**, 708-716.
Rixon,F.J. and Clements,J.B. (1982) *Nucleic Acids Res.*, **10**, 2241-2256.
Rixon,F.J., Campbell,M.E.M. and Clements,J.B. (1982) *EMBO J.*, **1**, 1273-1277.
Sandri-Goldin,R.M., Goldin,A.L., Holland,L.E., Glorioso,J.C. and Levine, M. (1983) *Mol. Cell. Biol.*, **3**, 2028-2044.
Smiley,J.R., Swan,H., Pater,M.M., Pater,A. and Halpern,M.E. (1983) *J.*

Virol., **47**, 301-310.
Stenberg,R.M., Thomsen,D.R. and Stinski,M.F. (1984) *J. Virol.*, **49**, 190-199.
Stinski,M.F., Thomsen,D.R., Stenberg,R.M. and Goldstein,L.C. (1983) *J. Virol.*, **46**, 1-4.
Svensson,C. and Akusjarvi,G. (1984) *EMBO J.*, **3**, 789-794.
Swanstrom,R.I. and Wagner,E.K. (1974) *Virology*, **60**, 522-533.
Tooze,J., ed. (1981) *DNA Tumour Viruses, 2nd ed.*, published by Cold Spring Harbor Laboratory Press, NY.
Watson,R.J. and Clements,J.B. (1980) *Nature*, **285**, 329-330.
Whitton,J.L., Rixon,F.J., Easton,A.E. and Clements,J.B. (1983) *Nucleic Acids Res.*, **11**, 6271-6287.
Wilkinson,G.W.G., Akrigg,A. and Greenaway,P.J. (1984) *Virus Res.*, **1**, 101-116.

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