Trans activation of plasmid-borne promoters by adenovirus and several herpes group viruses

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ABSTRACT
This paper describes experiments to test the ability of a number of viruses of the Herpes group, and also Adenovirus-2 and SV40, to activate transcription from the Herpes simplex virus-1 glycoprotein D and the rabbit β-globin promoters. Plasmids containing these genes were transfected into HeLa cells which were then infected with various viruses. Transcriptional activation in trans of the plasmid-borne promoters was monitored by quantitative S1 nuclease analysis of total cytoplasmic RNA isolated after infection. The results showed that Herpes simplex viruses 1 and 2, Pseudorabies virus, Varicella Zoster virus, Human Cytomegalovirus, Equine herpes virus-1 and Adenovirus-2 activate transcription from both promoters tested. In contrast, SV40 did not activate transcription in trans in this assay. The possible mechanisms of this activation are discussed.

INTRODUCTION
Herpes and Adeno virus gene expression is regulated to produce distinct classes of transcripts at different stages of infection (1-5; see ref 6 for reviews). The Immediate-Early (IE-) genes are transcribed first (7), and at least some of their IE-gene products are required for the activation of transcription from viral Early promoters (4,5,8). Recent investigation of the mechanism of this trans activation did not reveal sequences within a Herpes Simplex virus-1 (HSV-1) Early promoter that were a distinctive feature of such promoters and which were specifically required for HSV-1 IE-gene product activation (9,10). Maximum response to trans activation required all the promoter elements necessary for cis activation but no other sequences (9). This suggests that the mechanism of trans activation in this instance may be applicable to promoters other than those of HSV-1 Early genes. This suggestion is supported by the observation that the rabbit β-globin promoter can be
activated after transfection by infection with HSV-1 (10). In order to investigate the specificity of viral transactivation in more detail we have tested several Herpes viruses and Adenovirus-2 (Ad2) for their ability to activate during infection two different plasmid-borne promoters. We found that HSV-1, HSV-2, Pseudorabies virus (PRV), Equine abortion virus (EHV-1), Variella Zoster virus (VZV), Cytomegalovirus (CMV) and Ad2 (but not SV40) can activate transcription from both the HSV-1 glycoprotein D (gD) and rabbit β-globin promoters.

MATERIALS AND METHODS

1. Cells and Viruses.

HeLa cells were grown as described (9). Human Foetal Lung cells (HFL) and Monkey CV-1 cells were obtained from Flow Laboratories, Irvine, Scotland and grown according to their instructions. HSV-1 and HSV-2 were grown and titred in BHK C13 cells, PRV and Ad2 in HeLa cells, VZV and CMV in HFL cells, EHV-1 in rabbit kidney RK13 cells and SV40 in CV-1 cells.

2. Plasmids.

The plasmids used in these experiments are shown in Figure 1. pRED4 (10) and pβ(244+)β (11) have been described before. pβdel is a derivative of pβ(244+)β from which the 244bp Polyoma enhancer fragment has been deleted.

3. Calcium Phosphate Transfection, Infection of HeLa Cells, RNA Isolation and S1 Analysis.

These methods were as described (9,10). Viral infections were initiated 24hrs after transfection. In most cases the transfected HeLa, HFL or CV-1 cells could be infected simply by addition of a viral suspension to give a m.o.i. of 20 p.f.u. per cell. However, VZV grows as a cell associated virus, and is passaged as a stock of infected HFL cells. Thus infection of transfected HeLa cells in experiments using VZV was performed by addition of infected HFL cells. Therefore the precise time and multiplicity of VZV infection could not be determined. RNA was prepared at various times after infection as detailed in the figure legends. 10-20μg of total cytoplasmic RNA was hybridised to an end-labelled, single-stranded probe (Figure 1) and the resultant hybrids were treated with S1 nuclease and analysed on 8% acrylamide gels containing 7M urea as described (10).
RESULTS

Trans Activation of the HSV-1 Glycoprotein D Promoter in Short-Term Assays.

The initial approach was to determine whether different Herpes viruses could activate transcription from an HSV-1 Early promoter. Plasmid pRED4 contains the gD promoter fused to the rabbit p-globin coding region (10) while p(p244+)f contains two copies of the whole rabbit p-globin gene under the control of its own promoter and the Polyoma virus enhancer (11) (Figure 1).
FIGURE 2
Transcriptional activation in trans of the HSV-1 gD promoter by several viruses. Unless otherwise stated, HeLa cells were transfected with pRED4 and pλ(244+)φ and then infected with virus as noted, and RNA prepared at the indicated times after infection. Molecular weight standards are end-labelled HpaII fragments of pBR322. The bands at 160 and 147 bases which are closest to the sizes of the S1 hybrids are marked next to Figure 2(a) track 9. The positions of the gD/globin hybrid 5' ends from pRED4 and the φ-globin 5' ends from pφ(244+)φ and pδdel are shown here and in Figure 3 as gD and glob. (a) Transfection with pRED4 and pφ(244+)φ. Tracks 1 and 9; molecular weight markers. 2; RNA prepared 4hrs after infection with HSV-1 Glasgow strain 17. 3; HSV-2 strain HG52, 4hrs. 4; PRV, 6hrs. 5; EHV-1, abortogenic strain, 26 hrs. 6; VZV, 26hrs after addition of trypsinised HFL cells from an infected cell monolayer. 7; SV40, 26hrs. 8; uninfected. (b) All transfections included pRED4 and either pφ(244+)φ or pδdel as noted. Tracks 1 (pφ(244+)φ) and 2 (pδdel); RNA prepared 26hrs after infection with CMV strain AD169. Track 3; M.Wt. markers. Tracks 4 (pφ(244+)φ) and 5 (pδdel); RNA prepared 26hrs after infection with Ad2 in the presence of cytosine arabinoside at 50μg/ml. (c) Track 1; M.Wt. markers. Tracks 2 and 3; transfection of HFL cells with pRED4 and pφ(244+)φ or pδdel respectively. RNA prepared 76hrs after infection with CMV strain AD169.

Transcription from the gD promoter in pRED4 during short-term transfection experiments is entirely dependant on trans activation by IE-gene products (10). These plasmids were
transfected into HeLa cells, which were infected 24 hours later and RNA prepared at various times after infection (for details see Figure 2 legend). Transcription from the gD and \( \beta \)-globin promoters was analysed by S1 mapping of correctly initiated RNA using the end-labelled probe shown in Figure 1. The \( \beta \)-globin RNA indicated in Figure 2a, which derives from \( p\beta(244+)p \), acts as a control for each transfection (10).

The results show that several viruses strongly activate the gD promoter; HSV-1, HSV-2, PRV, EHV-1 and VZV all induced correctly initiated RNA (Figures 2a and 2b). This effect was detectable in most cases, depending on the speed of lytic cycle, early in infection. It was not possible to define the time of VZV infection accurately because the virus is cell associated; infection is not synchronous because it is initiated using infected cells. CMV did not appear to induce gD transcription in HeLa cells (Figure 2b track 1), which are not fully permissive for CMV replication. However, in permissive HFL cells, CMV did activate gD transcription (Figure 2c track 2). This implies either that the CMV IE-gene product requires for its activity a host factor not present in HeLa cells, or that CMV infection is blocked at an earlier stage in these cells. Preliminary evidence indicates that the former explanation is unlikely because co-transfection of a plasmid containing CMV IE-genes into HeLa cells leads to activation of the gD promoter (in preparation).

All the viruses tested above are members of the Herpes family. We decided to test unrelated viruses in this assay. Ad2 was chosen because mutants in its IE-gene, ElA, can be complemented by a PRV IE-gene product (12) which is thought to be equivalent to Vmw175 of HSV-1. In the presence of an inhibitor of DNA synthesis (to maintain the infection in an early phase (13)) Ad2 activated the gD promoter, but consistently less strongly than the Herpes viruses (Figure 2b track 4). This activation of gD transcription by Ad2 was reduced to very low levels if infection was allowed to proceed in the normal manner (results not shown). In contrast, SV40 did not activate the gD promoter in HeLa cells nor in permissive CV-1 cells (Figures 2a track 7 and 3c track 4). This implies that the SV40 large T-antigen does not act as a general transcriptional
FIGURE 3
Activation of the rabbit $\beta$-globin promoter in plasmids after short-term transfection and infection with various viruses. HeLa cells were transfected, unless otherwise stated. (a) Transfection with pRED4 and p$\beta$del. Tracks 1 and 7; M.Wt. markers. 2; RNA prepared 4hrs after infection with HSV-1. 3; HSV-2, 4hrs. 4; PRV, 6hrs. 5; EHV-1, 26hrs. 6; no infection. (b) Track 1; Transfection with pRED4 and p$\beta$del, RNA prepared 26hrs after infection with VZV infected HFL cells. Track 2; M.Wt. markers, the 160 and 147 bands are marked. (c) Tracks 1 and 2; transfection with pRED4 and P$\beta$(244+) or p$\beta$del respectively, RNA prepared 26hrs after infection with SV40. Track 3; markers. Tracks 4 and 5, Transfection of CV-1 cells with pRED4 and P$\beta$(244+) and p$\beta$del respectively, RNA prepared 26hrs after infection with SV40.

activator in these experiments. This conclusion is supported by the observation that the human $\beta$-globin promoter is not activated after transfection into Cos cells in which large quantities of large-T are present (14).

Trans Activation of the Rabbit $\beta$-globin Promoter During Virus Infection in Short-Term Assays.

It has been shown that the rabbit $\beta$-globin promoter is activated during HSV-1 infection when present in a plasmid (10). Therefore we tested whether infection with the viruses used above could simultaneously activate the rabbit $\beta$-globin
promoter. The Polyoma enhancer was removed from p\(\phi(244+)\phi\) to give p\(\phi\)del (Figure 1). Transcription of \(\beta\)-globin RNA from p\(\phi\)del is dependant on activation in trans (Figure 3a, compare tracks 2-5 with track 6). The transfection experiments in HeLa cells were repeated using pRED4 and p\(\phi\)del. In these experiments the gD 5' ends from pRED4 serve as the control since the extent of viral activation of this promoter is known (Figure 2). The results (Figure 3) indicate that all the viruses which activate the gD promoter also activate the rabbit \(\beta\)-globin promoter. The ratio of gD to globin 5' ends was similar in all infections; trans activation mediated by these viruses showed an apparent preference for the gD promoter. This probably reflects the intrinsic strengths of the promoters since similar differences were observed when they were activated in cis (9). CMV activated the \(\beta\)-globin promoter only in HFL cells (Figures 2b track 2 and 2c track 3) while SV40 infection did not activate this promoter (Figure 3c).

**DISCUSSION**

These results suggest that the mechanisms of trans activation induced by the Herpes and Adeno viruses may be similar. The absence of promoter specificity in these experiments is surprising as it could be argued that a virus would evolve specific control of its own genes. However, this analysis is supported by work in other laboratories. Trans activation of various related and unrelated promoters (different from those used here) mediated by the Adenovirus E1A gene product (13-17) has been demonstrated. The PRV IE-gene product can also activate unrelated promoters in transient assays (13,15). Also, no functional promoter sequences specific for trans activation of HSV-1 Early promoters have been detected (9).

All the above observations have been made in short term transfection experiments. However, in several studies using HSV transformed cell lines, integrated HSV-1 Early genes responded to superinfecting HSV-1 (18,19) although most cellular promoters do not appear to be activated during HSV-1 (20), or Adenovirus (22), infection. This apparent specificity may be the result of the cell lines being selected on the basis of expression of at
least one of the integrated HSV genes. Therefore, the integrated HSV promoters may be in an open portion of the chromosome accessible to trans activation. Indeed, we have recently demonstrated that promoters not related to HSV-1 may be activated by HSV-1 infection after they have been integrated into the host chromosome during biochemical transformation (in preparation). This suggests that similarly accessible host promoters may also be transiently activated during viral infection. For example, host heat-shock genes are activated during infection by Adenovirus, HSV-1 and HSV-2 (22-24). It remains to be seen whether other host genes are activated by viral infection and whether this has any relation to the oncogenic potential of these viruses.

The mechanism of trans activation of transcription in these experiments is not known. It is not an indirect consequence of viral infection per se because activation may be achieved by co-transfection of plasmids expressing viral IE-genes. The viral gene products responsible have been identified as those of the IE-genes of Adenovirus (ElA) and PRV (13,15) the HSV-1 IE-polypeptide Vmw175 (25 and unpublished data) and the corresponding IE-gene products of VZV and CMV (in preparation). It has not yet been possible to test for EHV IE-functions in this assay. Recognition of class specific DNA sequences in the promoter by the IE-gene products is unlikely (9), although interaction with promoter elements such as the TATA-box cannot be excluded. Other possibilities include modification of RNA polymerase II (B) or its associated transcription factors, modification (perhaps into a suitable chromatin structure) of the DNA template, localisation of the DNA in a transcriptionally active portion of the nucleus (perhaps the nuclear matrix (26)), inactivation of host encoded inhibitors (27) or release of RNA polymerase and other transcription factors from the host chromatin.

The mechanism of viral trans activation in the experiments reported here differs from that of some other documented cases, for example the Drosophila heat-shock genes (28), steroid hormone regulated genes (29,30) and the metallothionine gene (31). In these instances, DNA sequences
which are distinguishable from general promoter elements and which are specifically required for activation have been identified. Thus, activation of transcription may occur via factors which interact with these regulatory sequences whose presence may define families of co-ordinately regulated genes. In contrast, Herpes and Adeno virus Early gene regulation appears to be due to a different method of co-ordinate control. Further study of the mechanism of trans activation by viral IE-gene products should aid our understanding of the mechanism and regulation of eucaryotic gene transcription.

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