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Cellular gene induction during herpes simplex virus infection can occur without viral protein synthesis

L.M.Kemp, C.M.Preston*, V.G.Preston* and D.S.Latchman

Department of Zoology and Cell Biology, University College, London WC1E 6BT and *MRC Virology Unit, Institute of Virology, Glasgow G11 5JR, UK

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ABSTRACT

Infection of cultured cells with herpes simplex virus (HSV) results in the transcriptional induction of a small number of cellular genes. Although the majority of such genes are dependent upon viral protein synthesis for their induction, a small minority are not. These genes are induced by events occurring prior to the onset of viral protein synthesis, in particular by binding of the virus to the cell surface and cellular entry of the virion. The significance of such cellular gene induction early in viral infection is discussed in terms of virus-cell interaction in general and the mechanism of transformation by HSV in particular.

INTRODUCTION

The transcriptional activation of a small number of cellular genes in lytic infection appears to be a general feature of the DNA tumour viruses, occuring in viruses as diverse as SV40 (1) and the adenoviruses (2). The cases of cellular gene activation studied so far are dependent upon the synthesis in infected cells of viral trans-activator proteins such as SV40 large T or the adenovirus E1A protein, which in addition to acting on viral promoters (3,4), can also stimulate the transcription of some cellular genes (1,2). Interestingly the same proteins are also responsible for the ability of these viruses to immortalise cells of limited life span (5,6) and it has been suggested that this effect could be mediated via the constitutive induction of cellular genes involved in growth regulation (7).

Such a possibility is of particular interest in the case of herpes simplex virus (HSV) where transformation appears to take place via a 'hit and run' mechanism in which transient exposure to the virus produces a permanent effect on the host cell (8). Although lytic infection with this virus represses most host cell protein synthesis (9,10), a small number of cellular proteins do accumulate upon lytic infection (11) and at least one of these is over-expressed in cells transformed by HSV or other agents (12). By using an antibody to an HSV induced cellular protein (11) we have

recently isolated cDNA clones derived from the corresponding gene and shown that its transcriptional induction during lytic infection with HSV is dependent upon viral protein synthesis (13), paralleling the examples of cellular gene induction previously described for other viruses. To further study cellular gene induction by HSV, we have used differential screening techniques to isolate cDNA clones derived from other cellular genes which are induced by infection with this virus. Here we show that, although the majority of such genes, like the TI56 gene, are dependent upon viral protein synthesis for their induction, a small number are induced in the absence of such protein synthesis. These genes appear to be induced very early in lytic infection by binding of the virus to the cell surface or by cellular entry of the virion.

MATERIALS AND METHODS

Cells and Viruses Baby hamster kidney (BHK) cells clone 13 (14) and human foetal lung (HFL) fibroblasts (Flow Laboratories) were grown in Eagle's medium supplemented with 10% foetal calf serum. Infections using HSV-1 strain 17 (15) in the presence of cycloheximide were carried out using 200 µg/ml of the drug which was added with the virus and remained present throughout infection. Mock infected cells were treated similarly without virus addition. Infections with the HSV-1 mutant ts1204 (16) were carried out at the non-permissive temperature of 38.5 °C using viral stocks pre-warmed to this temperature. All infections were carried out at a multiplicity of 10 pfu/cell.

Plaque screening Stocks of recombinant phage were spotted in a regular array onto a lawn of growing E. coli and after overnight incubation to allow lysis to occur, replicate filters were taken, treated as described by Benton and Davis (17) and hybridised with labelled cDNA preparations.

RNA isolation and cDNA synthesis Polyadenylated cytoplasmic RNA was isolated from infected cells as previously described (13) and cDNA synthesized using the conditions described by Huynh et al (18) except that random hexanucleotides (Pharmacia) were used as primers.

Nuclear run-off assays Nuclear run-off assays were performed as previously described (13) and the labelled products used to probe replicate dot blots of recombinant plasmid or bacteriophage DNA.

RESULTS

In order to isolate cDNA clones derived from cellular genes induced during HSV infection, we prepared a cDNA library from HSV- infected BHK cell mRNA

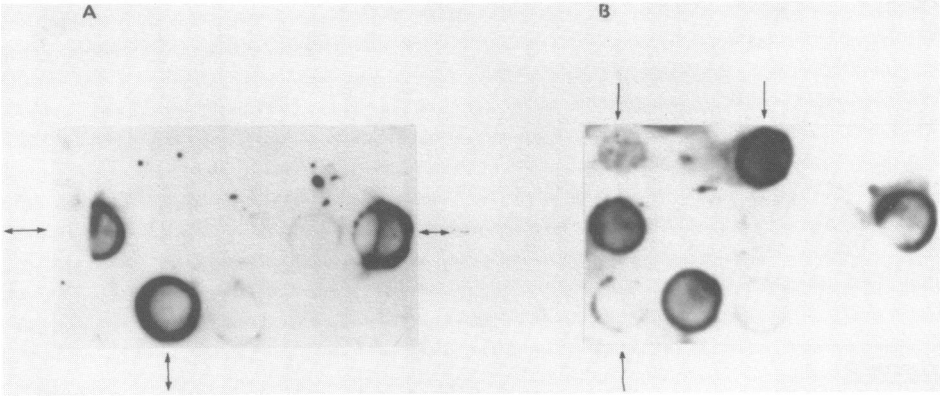

Figure 1
Screening of cDNA clones. Recombinant phage corresponding to cellular RNA species up-regulated upon viral infection were screened with cDNA prepared from mRNA of BHK cells either mock infected in the presence of 200 µg/ml cycloheximide (A) or infected with HSV-1 under similar conditions (B). Several clones (single headed arrows) show increased hybidization, that shown by clone 132 (top row, centre) being the most dramatic. Control clones derived from RNA species whose level does not change in normal infection are indicated by double headed arrows.

in the bacteriophage vector λ gt10(18) and carried out differential screening using RNA from mock-infected or virally-infected cells. This screening, the detailed results of which will be presented elsewhere (Kemp et al, submitted), resulted in the isolation of 56 clones hybridizing more strongly to the infected cell RNA and having no detectable homology to HSV DNA. These clones are therefore derived from cellular RNA species up-regulated upon infection with the virus.

To determine whether such up-regulation was dependent upon viral protein synthesis, we carried out infections in the presence of 200 µg/ml cycloheximide which completely prevented the synthesis of viral proteins as assayed by western blotting (data not shown). Labelled cDNA synthesised from the mRNA of cells either mock-infected or virally infected in the presence of cycloheximide was then used to screen replicate filters taken from bacteriophage plaques of clones derived from up-regulated RNAs. In these experiments two non-homologous clones (a4 and 31) showed increased hybridization equal to that seen in a normal infection whilst one clone (132) showed a more dramatic increase in a cycloheximide blocked infection than that seen in normal infections (Figure 1). Hence, the RNA species

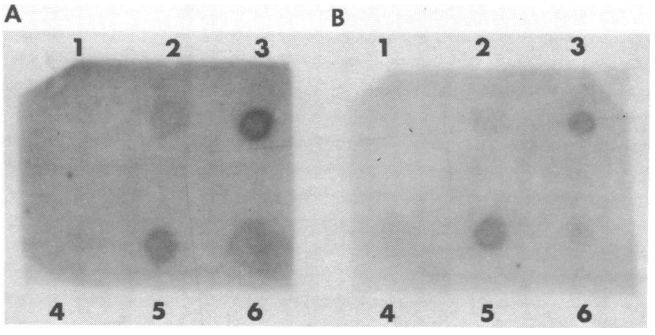

Figure 2
Nuclear run-off assays. DNA prepared from recombinant plasmids was spotted onto nitrocellulose and probed with 'run-off' products made from the nuclei of BHK cells either virally infected in the presence of 200 µg/ml cycloheximide (A) or mock-infected under similar conditions (B).
Key:- 1, pUC8; 2, Clone a4; 3, Clone 31; 4, Clone TI56/17; 5, Clone 123 (cDNA clone derived from an RNA species whose level does not change in normal infections); 6, Clone 132.

corresponding to these clones can accumulate during viral infection in the absence of detectable viral protein synthesis.

In order to investigate further the processes controlling such up-regulation, we prepared nuclei from cells either mock-infected or virally-infected in the presence of cycloheximide and carried out nuclear run-off assays to study the transcription of the cellular genes

Table 1 Results of nuclear run-off assays

CLONE	Mock infected in the presence of 200µg/ml cycloheximide	Infected with HSV-1 strain 17 in the presence of of 200µg/ml cycloheximide	Mock infected at 38.5°C	Infected with HSV-1 tsl204 at 38.5°C
TI56/17	0	0	0	0
a4	11	54	10	202
31	51	212	42	221
123	94	97	92	95
132	15	72	17	21

Figures are counts per minute binding to the indicated clone in hybridization with RNA synthesized by the various nuclei under run-off conditions, equal numbers of counts being used in each case. Figures are the average of two determinations, the background obtained with the plasmid vector pUC8 (approximately 10 cpm) has been subtracted in each case.

corresponding to clones a4, 31 and 132. The results of such assays (Figure 2 and Table 1) indicate that for all three clones studied, the up-regulation of their homolgous RNA species was mediated by increased transcription of the corresponding genes. In contrast, no increased transcription was detected using either a control clone (123) derived from an RNA species whose level is unchanged in infection or from the TI56 gene whose increased transcription in normal infection requires viral protein synthesis (13). Thus these experiments permit the novel conclusion that a small number of cellular genes can be transcriptionally induced during viral infection in the absence of viral protein synthesis.

This induction could be mediated by either of two events which occur prior to the onset of such synthesis, namely initial binding of the virus to the cell surface or subsequent membrane fusion and entry of the virion. To distinguish between such events we carried out infections at the non-permissive temperature with a temperature sensitive mutant of HSV-1, ts1204 (16). This mutant has been shown to bind to the normal cell surface receptor for HSV-1 but is defective in subsequent membrane fusion and internalisation. Screening of the cDNA clones with RNA derived from cells infected with this mutant (Figure 3) demonstrated that two clones (a4 and 31) continued to show increased hybridization under these conditions whilst clone 132 failed to do so. This result strongly suggests that the transcriptional induction observed in cycloheximde blocked infections for clones a4 and 31 is caused by the binding of the virus to the cell surface rather than by subsequent events. In agreement with this idea nuclear

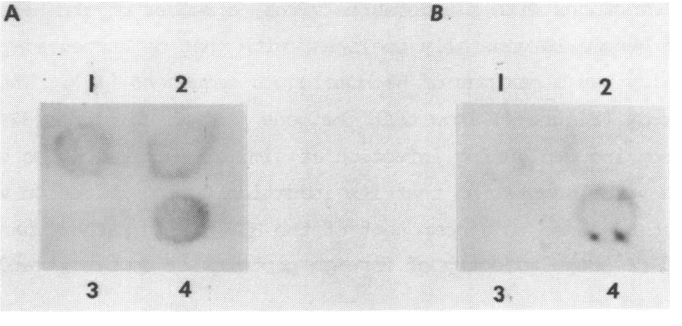

Figure 3
Screening of cDNA clones. Recombinant phage were screened using cDNA prepared from the mRNA of HFL cells either infected with ts1204 (A) or mock infected (B) at 38.5°C. Key:-1, Clone a4; 2, Clone 31; 3, Clone 132; 4, Clone 123 (control).

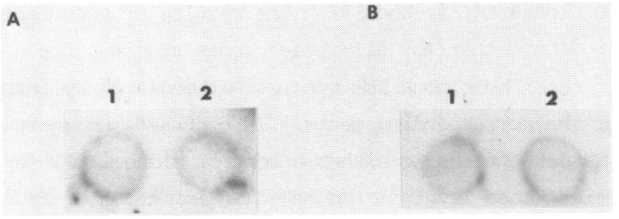

Figure 4
Screening of cDNA clones. Recombinant phage were screened with cDNA prepared from the mRNA of BHK cells either mock-infected (A) or infected with pseudorabies virus (B) in the presence of 200 ug/ml cycloheximide. Key:- 1, Clone 126 (cDNA clone derived from an RNA species whose level does not change in HSV infection); 2, Clone 132.

run-off assays carried out using nuclei from cells either mock-infected or infected with ts1204 at the non-permissive temperature (Table 1) confirmed that increased transcription of the genes corresponding to clones a4 and 31 could be detected in the infected cell nuclei.

In contrast no increased transcription or increased RNA levels corresponding to clone 132 could be detected in the ts1204 infected cells. The gene from which this clone is derived does not appear therefore to be induced by binding of the virus to the cell surface but rather by later events such as virion entry or uncoating prior to transcription. These events could involve for example, non-specific activation due to entry of large amounts of foreign protein and/or double stranded DNA or might be due to a more specific activation mediated by a component of the HSV virion. To study this question we investigated the behaviour of clone 132 during infections with pseudorabies virus, a member of the herpes virus family whose genome is generally co-linear with that of herpes simplex virus but which has a maximum of 8% homologous sequences (19). The results of such a study (Figure 4) show that the gene from which clone 132 is derived is not induced during infection at similar multiplicities with pseudorabies virus, suggesting that its induction during infection with HSV is a specific response to a component of the HSV virion rather than a non-specific response to entry of foreign proteins or double stranded DNA.

DISCUSSION

Previous studies reporting the activation of cellular genes in viral infection have been based on a small number of proteins known to accumulate upon infection with the virus (11,20). The results of such studies have

shown that the induction of the corresponding genes is dependent upon the synthesis of viral trans-activator proteins in the infected cell (1,13). In the experiments described here we have used a panel of cDNA clones derived from cellular genes induced by HSV infection to carry out a more extensive study of this phenomenon. These experiments showed that although the majority of these genes like those previously studied (1,2,13) were dependent upon viral protein synthesis for their induction, a minority were not. Hence, such genes represent a novel class of cellular gene whose activity can be regulated at the transcriptional level in the absence of viral protein synthesis. The novelty of this finding led us to study its mechanism.

Thus of the three genes induced in the absence of viral protein synthesis, two also showed induction during infections at the non-permissive temperature with the HSV-1 mutant ts1204 (16). This mutant has been shown to bind to the normal cellular receptor for HSV-1 but is unable to internalise the virion. The induction of these two genes in such infections therefore suggests that their transcription is stimulated by binding of the virus to its receptor. Such a conclusion is supported by the observation that one of the genes (a4) showed greater induction during infections with ts1204 where the virus remains bound to the cell surface compared with that seen in wild type infections where virus internalisation presumably leads to a loss of stimulation (data not shown).

Although the receptor for HSV has yet to be identified, that for Epstein Barr virus has been shown to be the complement receptor CR2 (21) whilst vaccinia virus binds to the receptor for epidermal growth factor (22) Hence, such receptors appear to be normal cell-surface components to which the virus can bind. It seems possible therefore that, by binding to its cellular receptor, the virus is mimicking the binding of the normal ligand for this receptor and hence causing the induction of cellular genes, normally induced by this binding. Further study of these genes and their protein products may throw light not only on their role in normal and virally-infected cells but also on the nature of the cellular receptor whose activation causes their induction.

In contrast to the behaviour of the other two clones, the gene from which clone 132 is derived although induced in cycloheximide blocked infections, showed no induction in ts1204 infection. Hence this cellular gene appears to be regulated at a stage subsequent to receptor binding but prior to viral protein synthesis, namely cellular entry or

uncoating of the virion. This gene showed a more dramatic induction in cycloheximide blocked infections than in normal infections (data not shown) exactly as occurs for the viral immediate-early genes whose transcription begins prior to viral protein synthesis and is super-induced when such synthesis is blocked with cycloheximide (23).

These findings raise the possibility that the gene from which clone 132 is derived is regulated by the same processes which stimulate immediate -early gene expression. Such stimulation has been shown to be dependent upon the action of Vmw65 a trans-activating protein present in the viral tegument of HSV-1 and HSV-2 (24). Interestingly, no such trans-activating protein can be detected in the pseudorabies virus virion (Campbell and Preston submitted) paralleling the lack of increase in clone 132 specific RNA observed in infections with this virus.

Such possible co-ordinate regulation of a cellular gene and the viral immediate-early genes, parallels the ability of the immediate-early gene products themselves to stimulate both the expression of cellular genes (13,25) and viral early genes (26). However, unlike the immediate early gene products which appear to act non-specifically on open promoters (27), induction by Vmw65 is dependent on the presence of a specific sequence (related to the consensus sequence TAATGARATTC, R = purine) up-stream from the stimulated gene (28), since Vmw65 does not bind directly to this sequence (CMP unpublished data) it may act by stimulating a normal cellular control system, increasing the efficiency of transcription of both viral immediate-early and some cellular genes having the consensus sequence.

Thus, the experiments described here permit the conclusion that the sequential activation of viral genes in lytic infection with HSV (for review see reference 29) is paralleled by the activation of specific cellular genes either because such genes have open promoters susceptible to immediate-early protein stimulation (27) or because such cellular genes are regulated by cellular control processes with which the virus interacts. Such activation of a minority of cellular genes coincides with a virally mediated repression of the majority of host cell functions which also occurs at a number of stages in infection notably virion entry (30) and synthesis of viral proteins (31).

These complex viral-cell interactions can eventually result in a variety of possible outcomes of infection including, in addition to cell lysis, both latent infections and transformation (for review see references 29,32). The observation that a small number of cellular genes are induced

by infection with HSV when most host cell protein synthesis is repressed, suggests that the products of such genes may play an important role in viral-cell interactions and in particular in determining the outcome of infection. If this is so such genes must be induced early on in infection, prior to commitment to the lytic cycle. Such a requirement is of particular importance in the case of HSV-induced transformation which can be achieved with UV-irradiated (33) or mutant viruses (34,35) capable of only the earliest stages of infection. Clearly the cellular genes described here which are induced early in the lytic cycle, by processes involving interaction with cellular systems, represent the best candidates for involvement in this process and, in agreement with this idea, we have already shown that RNA homologous to one of the clones described here (a4) is over-expressed in an HSV-transformed cell line compared with its primary cell parent (LMK and DSL unpublished data). We are currently using the clones described here to carry out more extensive expression studies in latently infected and transformed cells.

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