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Isolation of cDNA clones derived from a cellular gene transcriptionally induced by Herpes Simplex Virus

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ABSTRACT

A small number of cellular proteins accumulate to high levels in cells infected with Herpes Simplex Virus (HSV) despite a generalised repression of most host cell bio-synthesis. An antibody to one such protein has been used to screen a lambda gt11 library and for polysome immunoprecipitation in order to isolate cDNA clones derived from the corresponding gene. The cDNA clones have been used in dot blot and nuclear run-off assays to show that HSV, like other DNA tumour viruses can transcriptionally induce a cellular gene. The mechanism of this effect which is dependent on viral protein synthesis and its possible significance in transformation by HSV are discussed.

INTRODUCTION

The lytic infective cycle of DNA viruses such as the papovaviruses (SV40 and polyoma), the adenoviruses and the herpesviruses involves the sequential transcriptional activation of different groups of viral genes(1) such activation is mediated by viral proteins, made during the immediate early phase of infection, which induce the expression of other viral genes(2,3,4). In the case of both SV40 and adenovirus, these activators have also been shown to induce elevated transcription of specific cellular genes in normal infection (5,6). This effect of viruses as diverse as SV40 and adenovirus suggests that it may be of general importance in viral infection and it has been suggested that it is implicated in the transforming ability of these viruses(7).

If this is the case, such activation of specific cellular genes should also be demonstrable in infection with other DNA tumour viruses such as Herpes Simplex Virus, where the majority of cellular gene expression is strongly repressed by a variety of mechanisms including in at least one case, repression of transcription (8).

The transcriptional activator proteins of Herpes Simplex Virus Vmw 175 and Vmw 110 have been shown to induce the rabbit β globin gene in

co-transfection experiments (9) and in cell lines into which a β globin gene has been artificially introduced. However the endogenous β-globin gene is not activated in these experiments (10) indeed, no cellular gene in its normal environment has yet been shown to be transcriptionally activated by HSV infection. The induction demonstrated to date is thus non-specific acting on promoters in a particular configuration and failing to activate the same promoter within native chromatin. This non-specific activation is analogous to that mediated by the adenovirus E1A protein (11,12), which in addition activates specific cellular genes in normal infection (5), hence raising the possibility that such specific activation of a cellular gene could occur also in HSV infection.

A primary candidate for such an induced cellular gene is that encoding the protein recognised by monoclonal antibody TI56 which is a cellular protein present at low levels in all normal cells studied but which accumulates to high levels in cells infected with HSV (13). Here we report the isolation of cDNA clones derived from the gene encoding this protein (which we refer to as the TI56 gene and the TI56 protein respectively) and their use to demonstrate that the virus does transcriptionally activate this cellular gene.

MATERIALS AND METHODS

Cells

Baby Hamster Kidney cells (BHK) clone 13 (14) were grown in Eagle's medium with 10% new born calf serum and infected with HSV-2 strain 333 at a multiplicity of 20 pfu/cell. When infections were carried out in the presence of cycloheximide (50 µg/ml), the drug was applied at the time of virus infection and remained present until cells were harvested. For RNA, cells were harvested sixteen hours after infection. For preparation of nuclei, cells were harvested four hours after infection before the onset of any cytopathic effect and increased nuclear fragility. Hybridoma TI56 was grown in RPMI medium (13), with 10% foetal calf serum, culture supernatant was used in all experiments.

Antibody Screening

The λ gt 11 cDNA library was screened using culture supernatant from hybridoma TI56 as the first layer and peroxidase - conjugated rabbit anti-mouse immunoglobulin as the second layer (15).

Polysome Immunoprecipitation

Preparation of polysomes and immunoprecipiation were carried out

according to the method of Schneider et al(16) except that nascent polysomes synthesising the TI56 protein were purified on an immunadsorbent column of antibody TI56 coupled to activated sepharose.

Plaque Hybridization

Plaques isolated by antibody screening or polysome immunoprecipitation were tested for homology to TI56/17 by spotting out on a lawn of bacteria. After lysis had occurred DNA in the plaques was transferred to nitrocellulose by the method of Benton and Davis (17) and hybridized with oligo-nucleotide labelled insert (18) from clone TI56/17.

RNA Isolation

RNA was isolated from uninfected and infected cells by the NP40 lysis method (19) and after chromatography on oligo dT cellulose (20) was dot blotted as previously described (21).

Nuclear Run-off Assays

Nuclei prepared from uninfected and infected cells were allowed to continue RNA synthesis in the presence of ^{32}P labelled GTP under the conditions described by Greenberg and Ziff (22). After purification of labelled RNA it was used to probe replicate dot blots of various plasmid DNA samples. After hybridisation and washing individual 'dots' were cut out and counted.

RESULTS

Isolation of cDNA clones from uninfected cells

In order to isolate cDNA clones derived from the gene encoding the TI56 protein we used the antibody to screen a cDNA library derived from human T-cell mRNA, in the bacteriophage vector λ gt 11. In this vector, cDNAs are inserted into the phage β-galactosidase gene and hence a proportion will be expressed as fusion proteins with β galactosidase. Screening of two milion plaques from this library with the TI56 antibody resulted in the detection of thirty two positive plaques showing reactivity. Because the phage were plated at too high a density to allow individual plaques to be picked, the areas of the plate containing positive plaques were picked and subjected to secondary screening. Such secondary screening was carried out in two ways. As a preliminary, the picked plaques were spotted in a regular array on a lawn of growing E. coli and after lysis had occurred, screened with the antibody (Fig 1). This preliminary screen confirmed that nineteen of the thirty two picked regions did indeed contain immunoreactive plaques, and these were then plated out

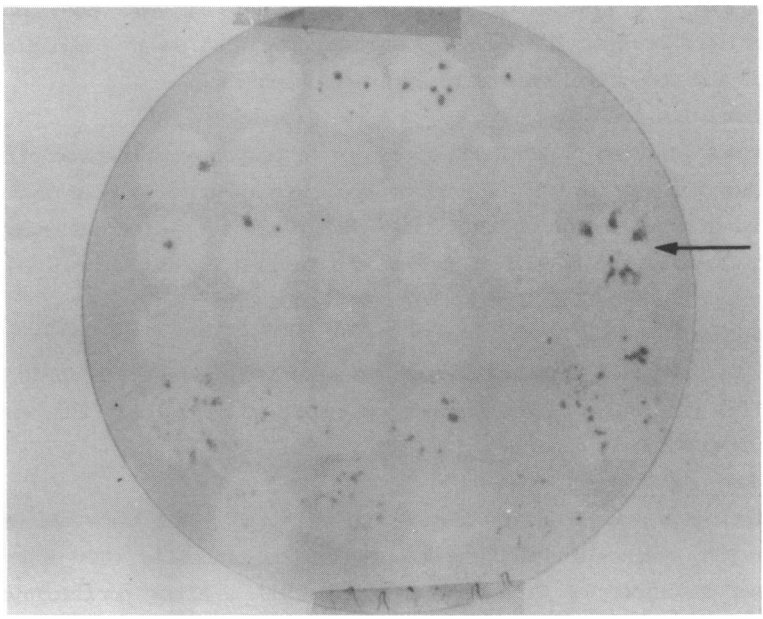

Figure 1
'Spot plaque' screening of primary positive regions with antibody TI 56. Thirty two positive regions from the primary screen were spotted and probed with antibody. Many of the spots show positive plaques, those obtained with clone 17 (used in subsequent analysis) are indicated by the arrow.

individually at low density in order to isolate them as single, clonally pure phage, reactive with the antibody (Fig 2).

If such phage do indeed contain inserts derived from the gene encoding the TI56 protein they should produce a β-galactosidase fusion protein larger than the native protein and reactive with the antibody. To test this prediction IPTG was used to induce the lac operon in bacteria lysogenic for these phage. One such lysogen derived from clone TI56/17 produces an inducible β-galactosidase fusion protein which is approximately 35 kd larger than the native form (Fig 3, tracks 9-12). This protein, unlike native β-galactosidase is reactive with the TI56 antibody (Tracks 3 - 6). Hence the phage isolated by this means contains a cDNA insert capable of encoding protein reactive with the TI56 antibody.

To test whether the clones isolated by this method were all derived from a single gene encoding the TI56 protein in uninfected cells, we purified the insert from clone TI56/17 by digestion with EcoRI and

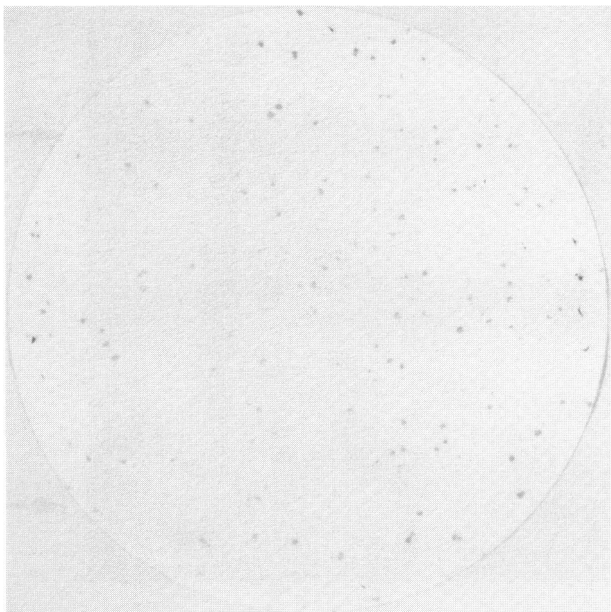

Figure 2

Low density screening of plaques derived from primary positive region 17. A number of the plaques show a positive reaction.

electrophoresis on low melting point agarose. The insert (which is approximately 600 base pairs in length) was labelled by oligonucleotide priming and used to screen all nineteen antibody positive phage. This experiment revealed strong homology of the insert to all the other antibody positive plaques (data not shown) indicating that all the cDNA clones are derived from a single gene encoding the TI56 protein synthesised in uninfected cells.

Isolation of cDNA clones from infected cells

In order to confirm that this gene is the same as that producing the high levels of protein accumulating in virally infected cells we used polysome immunoprecipitation to clone the gene from such cells. Polysomes prepared from Baby Hamster Kidney (BHK) cells (14) infected with Herpes Simplex Virus (HSV) Type-2 were passed down a sepharose CL4B column to which antibody TI56 had been bound. Both the fraction binding to the column and that passing through were then subjected to chromatography on

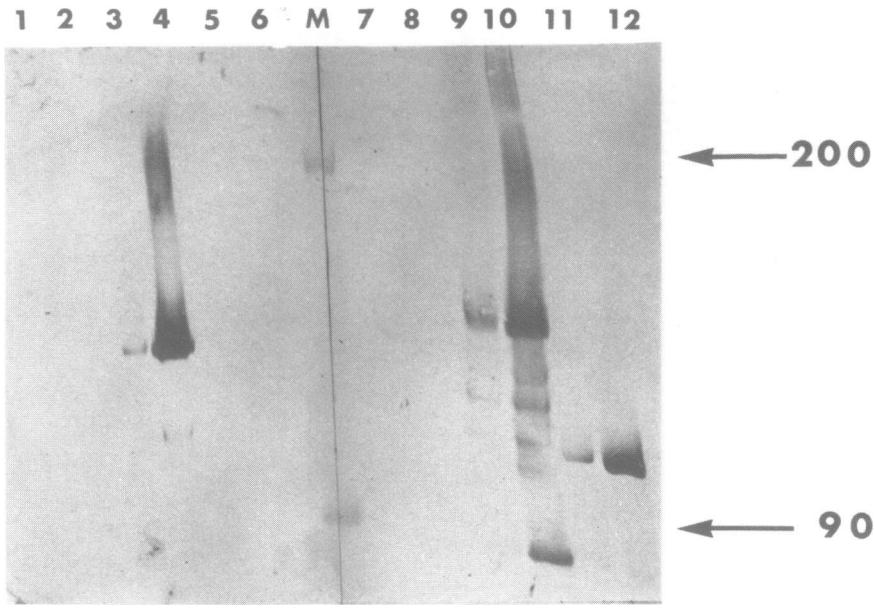

Figure 3

Western blots of lysogen samples run on a 5% polyacrylamide gel. The gel was divided along the marker track (M) and probed with either TI 56 antibody (Tracks 1 - 6) or anti-β-galactosidase antibody (tracks 7 - 12). Key:- Track 1 and 7 E. coli strain Y1089, Tracks 2 and 8 as 1 and 7 but induced with IPTG Tracks 3 and 9 E. coli strain Y1089, lysogenic for clone TI56/17, Tracks 4 and 10 as 3 and 9 but induced with IPTG, Tracks 5 and 11 E. coli strain Y1089 lysogenic for gt11, Tracks 6 and 12, as 5 and 11 but induced with IPTG. Track M - pre-stained molecular weight markers (BRL) of size 200 kd and 90 kd.

oligo dT cellulose in order to isolate fractions of polyA$^+$ RNA respectively enriched and depleted in the messenger RNA for the TI56 protein. Labelled cDNA was then prepared from both fractions and used to differentially screen a cDNA library prepared from HSV-2 infected BHK cells (Kemp et al, submitted). Four clones reacting strongly with the fraction enriched for the TI56 mRNA and not at all with the depleted fraction were isolated (not shown). Such clones, derived from the gene encoding the TI56 protein in infected cells showed no reaction with HSV-2 DNA, eliminating the possibility that the accumulation of immunoreactive protein in infected cells was due to cross-reactivity of the antibody with a viral protein. Moreover, the clones reacted strongly with labelled insert purified from clone TI56/17 which had been isolated from non-infected cells (Fig 4)

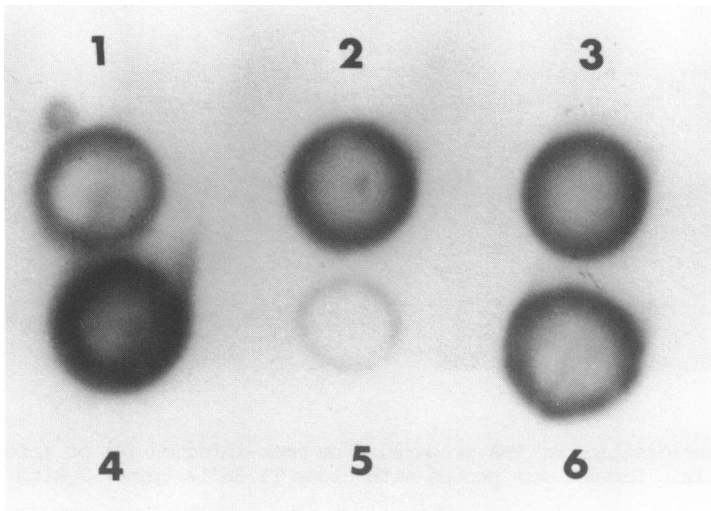

Figure 4
Screening of plaques, isolated by polysome immunopreciptation, for homology to clone TI56/17.
Key:- 1,2,3 and 6:- polysome clones 1-4; 4:- clone TI 56/17; 5: unrelated cDNA clone (hsp90) as control.

indicating that the same or very closely related cellular genes encode the low level of protein in normal cells and the high level accumulating in virally infected cells.

RNA homologous to the TI56 cDNA clones accumulates upon infection

The availability of cDNA clones derived from this gene permits the assessment of the level at which the virus induces accumulation of the protein. To this end we isolated messenger RNA from HSV-2 infected BHK cells and parallel cultures which had been mock-infected without virus. The two RNA samples were quantitated both by poly U assay (23) and by hybridisation with a probe (Clone 123) homologous to a high abundance RNA species whose level is unchanged upon HSV-2 infection (Kemp et al, submitted). Equal amounts of RNA were then spotted onto nitrocellulose and probed with the insert from clone TI56/17. The clone detected a low level of RNA in the uninfected sample which was greatly increased upon infection (Fig 5). Such behaviour which exactly parallels that of the TI 56 protein is in contrast to that of a number of cellular RNA species which are repressed upon infection (24,25).

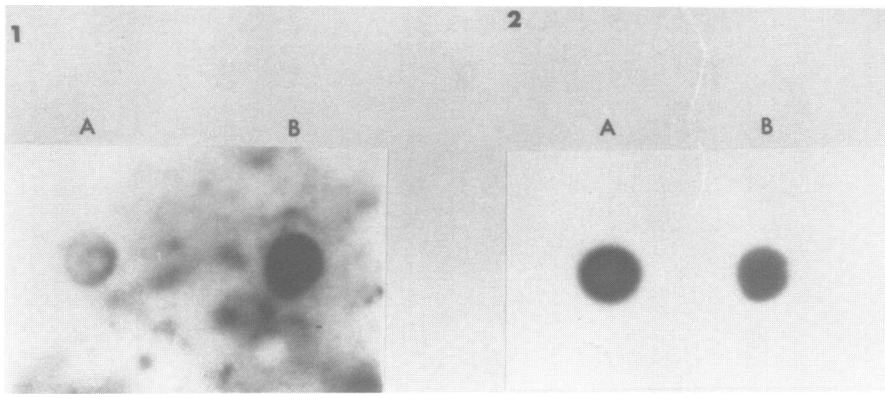

Figure 5
Dot blot hybridisation of RNA prepared from mock-infected (A) or infected (B) BHK cells. Panel 1 was probed with clone TI 56/17, panel 2 with clone 123.

The TI56 gene is transcriptionally induced by HSV infection

The finding that the TI56 RNA accumulates upon infection eliminates mechanisms such as selective translation or stabilisation of the TI 56 protein by association with virus protein as explanations of the increased protein accumulation in infected cells. However, as well as increased transcription, a variety of other mechanisms such as improved processing or stability of the RNA could mediate the increase in RNA level. In order to test whether the TI56 gene was indeed transcriptionally induced upon infection we performed nuclear run-off assays using nuclei prepared from parallel samples of HSV-2 infected and mock infected quiescent BHK cells. The labelled products of such assays were then used to probe filters onto which various DNA samples had been spotted and after washing individual spots were counted. The count obtained in this way is a measure of the rate of transcription of the particular gene in nuclei from which the sample is derived. The results of such assays (Table 1) indicated that although the level of TI56 gene transcription in quiescent uninfected cells was too low to be detected by this technique, such transcription was readily detected in infected cell samples indicating that the TI56 gene is transcribed at an increased level after infection. Such increased transcription was in contrast to the apparently decreased level of transcription observed for the control clone whose RNA level remains the same on HSV-2 infection. This may be due to a real decrease in the level

TABLE I

Results from nuclear run-off assays.

Clone	Source of nuclei: Uninfected BHK cells	Infected BHK cells	BHK cells treated with cycloheximide	BHK cells infected in the presence of cycloheximide.
TI 56/17	0	52	0	0
123	120	95	110	102

Figures are counts per minute binding to the indicated clone in hybridisation with RNA synthesised by the various nuclei under 'run-off' conditions. Figures are the average of three separate determinations, the background obtained with the plasmid vector pUC13 (approximately 10 cpm) has been subtracted in each case.

of transcription of this gene which is not immediately reflected in the level of its (presumably stable) RNA or may simply reflect a decreased efficiency of the nuclear run-off assay itself in infected nuclei. In the latter case, the increase in TI56 transcription may actually be greater than that which we have observed.

In order to determine the mechanism by which transcription of the TI56 gene is induced by the virus we carried out viral infections in the presence of cycloheximide which will prevent the synthesis of viral proteins. Nuclei were isolated from cultures infected or mock infected in the prescence of cycloheximide and used in nuclear run-off assays as before. Under these conditions no induction of TI56 transcription could be detected (Table 1) although transcription from the control clone was observed as before. Hence the transcriptional induction of the TI56 gene during viral infection requires the synthesis of viral protein(s). Such a requirement exactly parallels that previously observed for the TI56 protein which is not induced under immediate early conditions (cycloheximide/actinomycin D reversal - NLT unpublished data).

DISCUSSION

By isolating cDNA clones from a cellular protein up-regulated by HSV infection we have shown that the gene encoding this protein is

transcriptionally induced by HSV and that viral protein must be synthesised in the infected cell for this effect to occur. This finding parallels previous findings with a wide range of other DNA viruses such as SV40(6) and adenovirus (5) in which this effect has been demonstrated. This suggests that the specific activation of particular cellular genes is a common effect of all DNA viruses whether like SV40 they generally stimulate cellular metabolism (1) or, like HSV, produce a very strong repression of host cell functions (25).

In the case of HSV the mechanism by which the activation of a few genes occurs against a background of generalised repression is unclear. The viral immediate early proteins Vmw 175 and Vmw 110 are capable, like the adenovirus E1A product, of non-specifically activating cellular genes such as β-globin in co-transfection experiments(9) or where such genes have been artificially introduced into cells (10). The non-activation of the endogenous β-globin genes in such experiments suggests that an open chromatin structure may be important in this non-specific activation.
Thus the cellular genes activated in normal infection might be those with accesible promoters (10). This idea is in agreement with the observation that that the heat shock genes whose protein products accumulate in infections with both mutant (26) and wild-type (LaThangue and Latchman, submitted) HSV have open promoters in normal cells (27). Hence, non-specific activation of promoters in a particular chromatin structure could give the observed specificity of induction. However, the observation that viral immediate-early protein Vmw175 can stimulate viral early gene promoters whilst repressing its own promoter (28) suggest that this protein at least, does possess a measure of sequence specificity for particular promoters. Such sequence specificity could clearly produce specific activation of cellular genes having sequence homology to viral promoters.

Whatever the nature of HSV induced gene activation, a consideration of the nature of the genes which are induced suggests that this effect is of functional significance in the infected cell. Thus the fact that the TI56 protein is also induced by heat shock (13) suggests that its induction in viral infection is part of a generalised stress response which also produces accumulation of the heat shock proteins hsp70 (26) and hsp90 (La Thangue and Latchman, submitted). The most obvious explanation for this induction of stress proteins is that they represent an attempt by the host cell to defend itself against viral infection. However, the possibility remains that the virus may induce cellular functions which play some role

in the lytic cycle such as mediating the repression of cellular biosynthesis also seen in heat shock (29).

These considerations suggest that the transcriptional induction of host cell proteins is likely to be of importance in viral-cellular interactions occuring in viral infection which, in the case of HSV can result in lytic or latent infections or in transformation (30). Indeed the observation that a wide variety of DNA viruses with the potential for cellular transformation can induce cellular genes has led to the suggestion that at least the immortalisation step of such transformation takes place via the activation of cellular genes involved in growth control (7). The activation of the TI56 gene by HSV is particularly relevant in this regard in that the protein encoded by this gene is synthesised in a growth regulated manner being over-expressed in exponential compared to confluent cells (13).

Such a finding is also of particular importance in that the mechanism by which HSV can transform cells is unclear. Thus although the virus is involved in the initiation of transformation the observation that no viral DNA need be retained in the transformed cell line (31) has led to the suggestion that the virus transforms via a hit and run mechanism in which transient exposure to the virus produces a permanent effect on the host cell (32). Our previous observations that cells tranformed by HSV contain elevated levels of proteins induced during normal infection (32, LaThangue and Latchman, submitted) when taken together with our current demonstration that like other transforming viruses HSV can transcriptionally induce cellular genes, suggests a mechanism by which such transformation could occur.

The considerations discussed above suggest that a study of the nature of the viral proteins mediating cellular gene induction and the role the products of these genes play in virally-infected and transformed cells would prove fruitful. We are currently using the clones derived from the TI56 gene which we have isolated in order to carry out such a study.

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