The EMBO Journal vol.3 no.2 pp.267–277, 1984

Herpes simplex virus infection causes the accumulation of a heat-shock protein

N.B.LaThangue*, K.Shriver[1], C.Dawson and W.L.Chan

Imperial Cancer Research Fund, Tumour Immunology Unit, Department of Zoology, University College London, Gower Street, London WC1E 6BT, UK, and [1]Genetic Systems Corporation, 3005, First Avenue, Seattle, WA 98121, USA

*To whom reprint requests should be sent
Communicated by M.Raff

A monoclonal antibody, produced from mice immunized with a herpes simplex virus (HSV)-infected cell extract, reacts with a molecule which is present in uninfected cells and which accumulates in large amounts during HSV 2 infection. In uninfected cells this molecule is growth regulated, in that exponentially growing cells have intense nuclear immunofluorescence, whereas confluent quiescent cells have little. It has a mol. wt. of 57 000 (p57) in exponential cells, and one of 61 000 (p61) in quiescent cells. In HSV 2-infected cells, p57 accumulates and nuclear and cytoplasmic immunofluorescence increases. In uninfected cells, p57 also accumulates during heat-shock treatment, and this is associated with a new immunofluorescence throughout the cytoplasm. We suggest that HSV 2 infection induces a cellular stress response which is involved in the shut-off of host cell polypeptide synthesis.
Key words: herpes simplex virus infection/heat-shock protein/monoclonal antibody

Introduction

Cell-encoded proteins have a critical role in determining the effect that a virus exerts on the host cell. This is well established for the RNA tumour viruses, where transformation appears to be dependent on the abnormally high expression of genes related to normal cellular genes (Bishop, 1981; Hayward *et al.*, 1981), whereas for some DNA tumour viruses it may involve stabilisation of a host cell nuclear protein (Lane and Crawford, 1979; Luka *et al.*, 1980; Sarnow *et al.*, 1982). Other viruses have been found to induce heat-shock or 'stress' proteins (Nevins, 1982; Collins and Hightower, 1982; Khandjian and Türler, 1983). The function of the stress proteins remains unknown, although they may be involved in a protective response.

Herpes simplex viruses (HSV) are large DNA viruses with the potential to encode many polypeptides (Haarr and Marsden, 1981). *In vitro,* virus penetration into the cell usually results in a lytic productive infection, which is associated with an early shut-off in host cell-directed macromolecular synthesis (Sydiskis and Roizman, 1966; Nishioka and Silverstein, 1977; Fenwick and Walker, 1978; Hill *et al.*, 1983). The mechanisms of this shut-off remain obscure although a virion component (Fenwick and Walker, 1978) and an early gene product (Read and Frenkel, 1983) may be involved. Productive infection is not the only outcome; in neuronal cells both HSV 1 and HSV 2 may remain latent (Baringer, 1974) while HSV 2 has been implicated in transformation and oncogenesis (Galloway and McDougall, 1983). This variability in response to HSV infection suggests that host cell factors determine the outcome of the virus-cell interaction.

To understand how genes are regulated during HSV infection we have prepared monoclonal antibodies reacting with affinity purified DNA-binding proteins (DBP) from infected cells (LaThangue and Chan, 1983). Here we report on one

Table I. Summary of monoclonal antibodies used in the present study

Antibody designation	Antigen (mol. wt.)
TG18	155 000 HSV DBP[a]
TG102	115 000 HSV glycoprotein[a]
E9F10	120 000[b] major HSV DBP
TGH3	Host cell DBP[c]
H4	Raji cell DBP

[a]Previously characterised (LaThangue and Chan, 1983).
[b]Shriver, in preparation.
[c]LaThangue, unpublished.

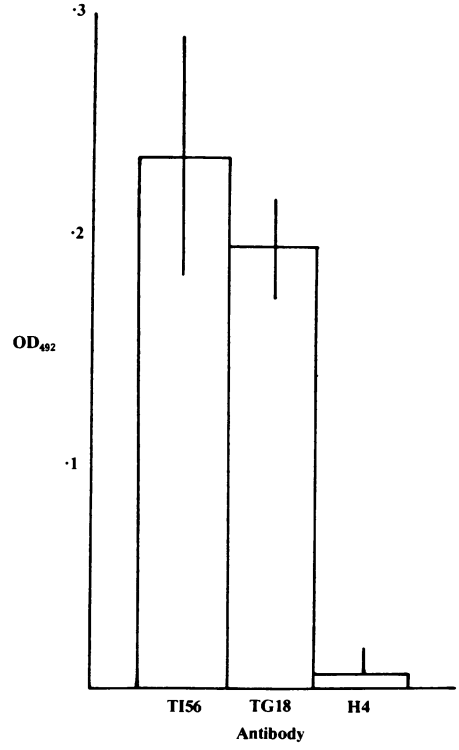

Fig. 1. Solid phase enzyme immunoassay of T156 binding to DBP. Monolayers of BHK 21 cells were infected with HSV 2 strain 333 and harvested at 15 h p.i. Infected cells were solubilised and DBP purified by affinity chromatography with DNA-cellulose. The eluted DBP were used as antigen at a concentration of 2.0 μg/well, and the immunoassay was performed as described. Antibody TG18 served as a positive control, and antibody H4 as a negative control.

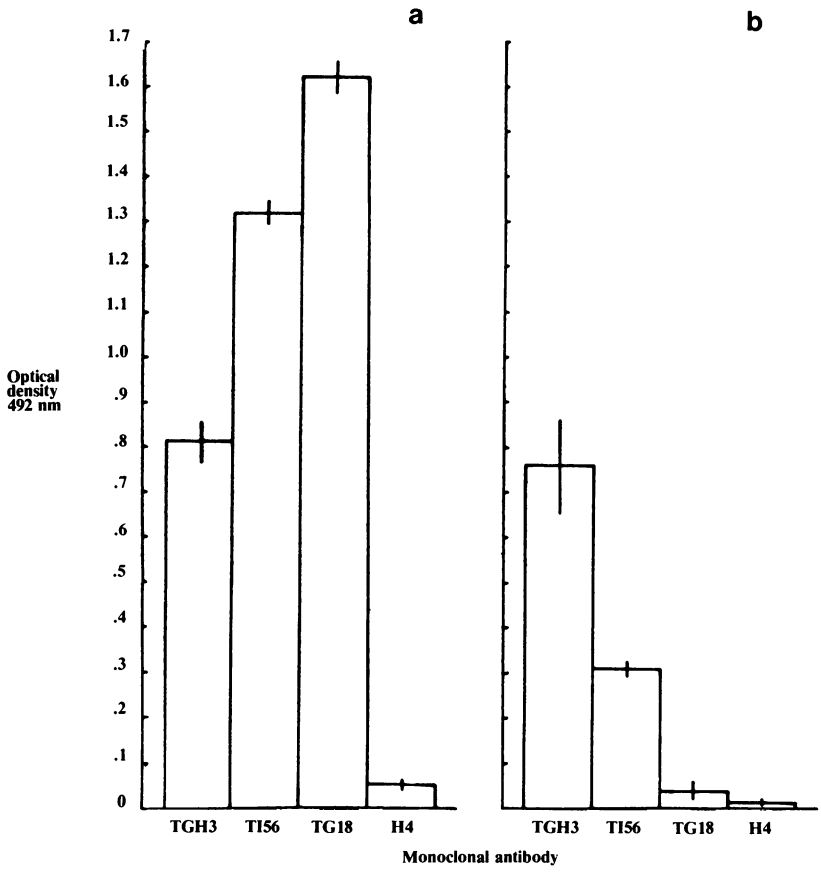

Fig. 2. Solid phase enzyme immunoassay of TI56 binding to HSV 2- and mock-infected cell extracts. Monolayers of BHK 21 cells were infected and solubilised as described in Figure 1 and Materials and methods. Antigen was used at 5 μg/well; the antibodies were used as in Figure 1; TGH3 reacts with a host cell DBP whose concentration is not altered by HSV 2 infection. Binding to **(a)** HSV 2-infected cell extract **(b)** mock-infected cell extract.

such antibody, TI56, which reacts with a host cell molecule. In uninfected cells, the latter is located within nuclei where its concentration is growth regulated, while in HSV 2-infected cells it begins to accumulate early after infection, to reach a concentration much greater than that in uninfected cells. In addition, this molecule accumulates in uninfected cells which have been subjected to heat shock (Ashburner and Bonner, 1979). This is the first report on a host cell molecule which accumulates within wild-type HSV 2-infected cells. Because this molecule also accumulates within heat-shocked cells, we suggest that it functions in the shut-off of cellular gene expression which occurs in HSV-infected cells.

Results

Production of anti-DBP monoclonal antibodies

Hybridoma TI56 was produced as described (Howes *et al.*, 1979). Hybridomas TG18 and TG102, described previously (LaThangue and Chan, 1983), react with a virus-encoded 155 000 mol. wt. DBP and a virus-encoded glycoprotein (probably gB), respectively. Antibody H4 was produced from mice immunized with DBP from the Epstein-Barr virus-transformed Raji cell line and was used as a control antibody throughout. Antibody E9F10 recognises the major HSV 2 DBP (K.Shriver, in preparation) and was produced as described (Goldstein *et al.*, 1983). Antibody TGH3 recognises a host cell DBP (LaThangue, unpublished). The antigens defined by these antibodies are summarised in Table I.

Antibody TI56 reacts in an enzyme immunoassay which used as antigen DBP purified from HSV 2-infected BHK 21 cells, by calf thymus DNA-cellulose affinity chromatography (Figure 1). In this assay, antibody TG18 served as a positive control and antibody H4 as a negative control.

Another enzyme immunoassay using antigen prepared from HSV 2 and mock-infected BHK 21 cells showed that antibody TI56 binds to mock-infected cell extracts (Figure 2b). There was increased binding with the infected cell extract (Figure 2a), which was comparable with that for antibodies reacting with virus-encoded molecules (as illustrated by antibody TG18). Antibody TGH3 recognises a host cell DBP whose concentration was not altered by HSV 2 infection. This assay suggests that antibody TI56 recognises a host cell molecule which accumulates within HSV 2-infected cells. Since it was not clear whether the antibody reacts with a common epitope on two different molecules (encoded by virus or cell), or alternatively with the same molecule in both situations, we characterised the molecule by immunofluorescence and mol. wt.

Characterisation within uninfected cells

The intracellular location of the TI56-defined molecule was studied by indirect immunofluorescence. Human embryo fibroblasts (Flow 5000) were used since their growth could be easily controlled. In exponentially growing cells, an intense granular nuclear immunofluorescence was seen, with little staining within the cytoplasm (Figure 3a). The intensity of the staining varied from cell to cell, and within exponentially growing cultures some nuclei were completely negative. Most

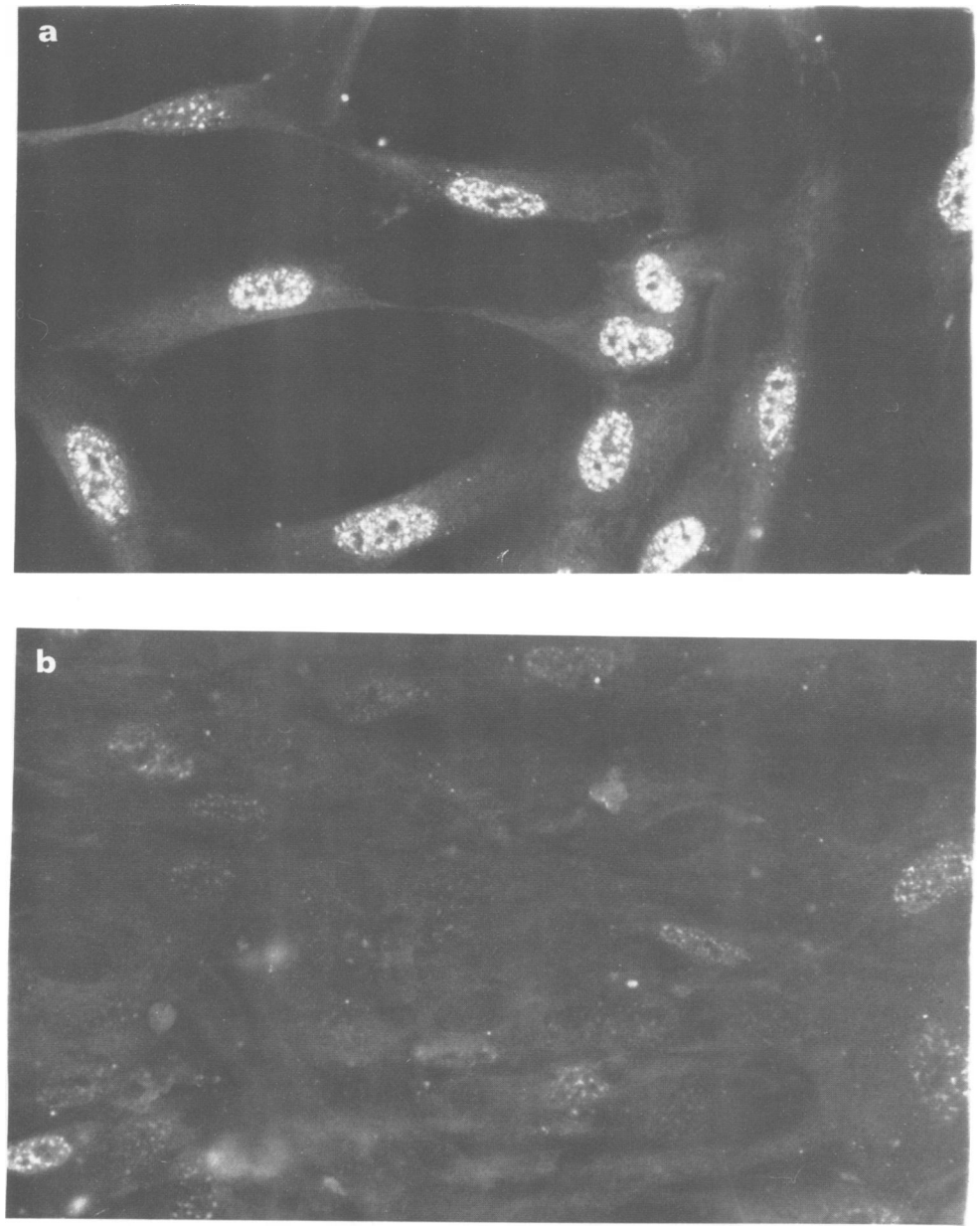

Fig. 3. Immunofluorescence with antibody T156 on uninfected cells. Human embryo cells were grown on sterilised coverslips and stained as described, **(a)** during exponential growth, **(b)** 24 h after reaching confluence. There was no fluorescence with a control antibody (not shown). Magnification 630x.

nuclei were negative in quiescent confluent cultures (Figure 3b). This indicates that the molecule is growth regulated, and accumulates during rapid growth. Other immunofluorescence studies have shown similar characteristics within a wide variety of other cell lines of diverse origin, and that the nuclear accumulation occurs during late G1 and S phase of the cell cycle (LaThangue and Chan, in preparation).

The T156-defined molecule was further characterised by radio-immunoprecipitation. Since its accumulation was growth regulated, BHK 21 cells were radiolabelled at three different stages of growth: (i) during exponential growth; (ii) at confluence where the cultures contained roughly equal numbers of cells with and without the nuclear immunofluorescence characteristic of exponential cells; and (iii) post-confluence, where most cells contained no, or a low level of nuclear immunofluorescence (Figure 3b). After radiolabelling, cells were solubilised in RIPA buffer and used for immunoprecipitation. From exponential cell extracts, antibody T156 immunoprecipitated one predominant polypeptide of 57 000 mol. wt., p57 (Figure 4, track 2); in some immunoprecipitations minor lower mol. wt. polypeptides were apparent. From confluent cells of condition (ii) it immunoprecipitated p57 and an additional polypeptide of 61 000 mol. wt., p61 (Figure 4, track 4), while from confluent cells of condition (iii) it immunoprecipitated a major polypeptide of 61 000 mol. wt., p61 (Figure 4, track 6).

These results indicate that antibody T156 recognises a molecule whose nuclear accumulation is growth regulated, and that small differences in its mol. wt. exist which depend on the growth state of the cells; p57 could be precipitated from exponential cells and p61 from quiescent confluent cells. These small mol. wt. differences could result from a post-translational modification of the molecule, such as phosphorylation, although products of different genes whose ex-

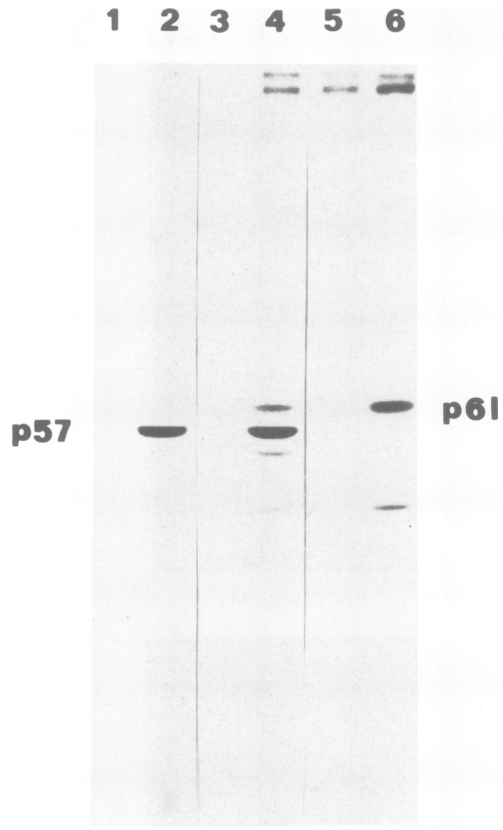

Fig. 4. Radioimmunoprecipitation from uninfected cells with antibody TI56. BHK 21 cells were radiolabelled with [^{35}S]methionine at three stages of growth, solubilised in RIPA buffer and immunoprecipitated with either TI56 (**tracks 2, 4** and **6**) or H4 (**tracks 1, 3** and **5**). Cells were radiolabelled **(a)** during exponential growth (**tracks 1** and **2**), **(b)** when they had just reached confluence (**tracks 3** and **4**) and **(c)** 24 h after reaching confluence, that is, post-confluence (**tracks 5** and **6**). This autoradiograph results from an 8-day exposure to the processed gel.

pression is growth regulated cannot be excluded. The results of this immunoprecipitation experiment cannot be interpreted quantitatively as the cell extracts were not adjusted for cell density.

Characterisation within HSV 2-infected cells

When confluent human embryo fibroblasts were infected with HSV 2, an increased nuclear immunofluorescence was seen within 2 h which increased progressively until, by 6 h post-infection (p.i.), an intense nuclear staining was seen, which had a continuous rather than granular appearance (Figure 5a). By 6 h p.i., the virus was replicating in most cells, as shown by immunofluorescence staining with antibody E9F10 (Figure 5b). At 9 h p.i., more nuclei had the characteristic staining pattern produced by antibody TI56 and by 16 h p.i. immunofluorescence was seen throughout the cytoplasm and nucleus of human embryo fibroblasts, when small intensely stained discrete structures could be seen (Figure 5c,d); in BHK 21 cells at 16 h p.i. the fluorescence was mostly nuclear. Mock-infected confluent quiescent cultures showed no increase in TI56 staining at any time (not shown). These observations suggest that the concentration of the TI56 molecule increases as HSV 2 replication progresses.

To determine whether the TI56 molecule that accumulated in the nucleus and cytoplasm of HSV 2-infected cells was the same as that found in the nucleus of uninfected cells, we characterised it by size. Confluent monolayers of BHK 21 cells were infected and radiolabelled until 16 h p.i. The cells were then harvested, solubilized in RIPA buffer and immunoprecipitated with four different antibodies: TI56 and three antibodies which served as positive controls for virus gene expression – TG102, TG18 and E9F10. Antibody TI56 immunoprecipitated a series of polypeptides with mol. wts. ranging from 46 000 to 57 000 (Figure 6a, track 4). The three anti-virus antibodies reacted with the expected polypeptide: TG102 with gB, TG18 with 155 000 mol. wt., and E9F10 with major DBP (Figure 6a, tracks 1, 2 and 3, respectively). There was no detectable immunoprecipitate from mock-infected cells on this autoradiograph although with longer exposure an immunoprecipitated polypeptide was detectable (not shown). The mol. wt. analysis, and the enzyme immunoassay and immunofluorescence data suggest that the TI56-defined molecule is present in HSV 2-infected cells at much greater concentrations than in uninfected cells. Although the TI56 molecule varies qualitatively with cell growth (Figure 4), the similar mol. wts. of the polypeptides in HSV 2-infected and uninfected cells suggests that the same molecule is recognised in both situations. Further evidence for this came from *Staphylococcus aureus* V8 protease peptide mapping (Cleveland *et al.*, 1977). For this, the TI56-specific molecules were purified from RIPA extracts of HSV 2- and mock-infected confluent cells, using an immunoadsorbent column prepared from antibody TI56 (Chan, 1983; LaThangue and Chan, 1983). The immunoadsorbent-purified molecules were then radiolabelled with [^{125}I]iodine and electrophoresed in a polyacrylamide gel. The p57 polypeptides were located, cut out, and digested with V8 protease as described. Figure 7 shows the results of such an experiment. From the peptide digests, it is clear that the p57 polypeptides in HSV 2- and mock-infected confluent cells are related. This indicates that p57 increases in concentration within HSV 2-infected cells. In HSV 2-infected cells, the lower mol. wt. polypeptides (Figure 6a, track 4) may be precursors to p57 which are post-translationally modified, but we cannot exclude products of a family of genes.

Immunoblotting was used to determine at which time this increased concentration could first be detected (Figure 6b). Confluent BHK 21 cells were infected with HSV 2, harvested at intervals p.i. and analysed by immunoblotting. There was no detectable binding to SDS extracts from mock-infected cells (Figure 6b, track 1), probably reflecting the low concentration of this molecule in uninfected confluent cells or a charge difference. However, binding to uninfected cells can be demonstrated using RIPA extracts (not shown), suggesting it is caused by a concentration effect. At 2 h p.i., antibody TI56 recognised a polypeptide of 46 000 mol. wt. (Figure 6b, track 2), which increased in concentration with time. At 6.5 h p.i., polypeptides ranging from 46 000 to 57 000 mol. wt. were recognised (Figure 6b, track 4), and these had a similar mol. wt. to those immunoprecipitated from HSV 2-infected cell extracts (Figure 6a, track 4). Thus a TI56-specific polypeptide can first be detected at 2 h p.i. and its concentration, together with that of others of greater mol. wt., increases with time. This is compatible with a hypothesis that the lower mol. wt. polypeptides are precursors to p57.

Characterisation as a heat-shock protein

Exponential cultures of human embryo fibroblasts were heat-shocked for 30 min at 46°C and then studied by immunofluorescence with antibody TI56. There was an increased immunofluorescence within the cytoplasm which was

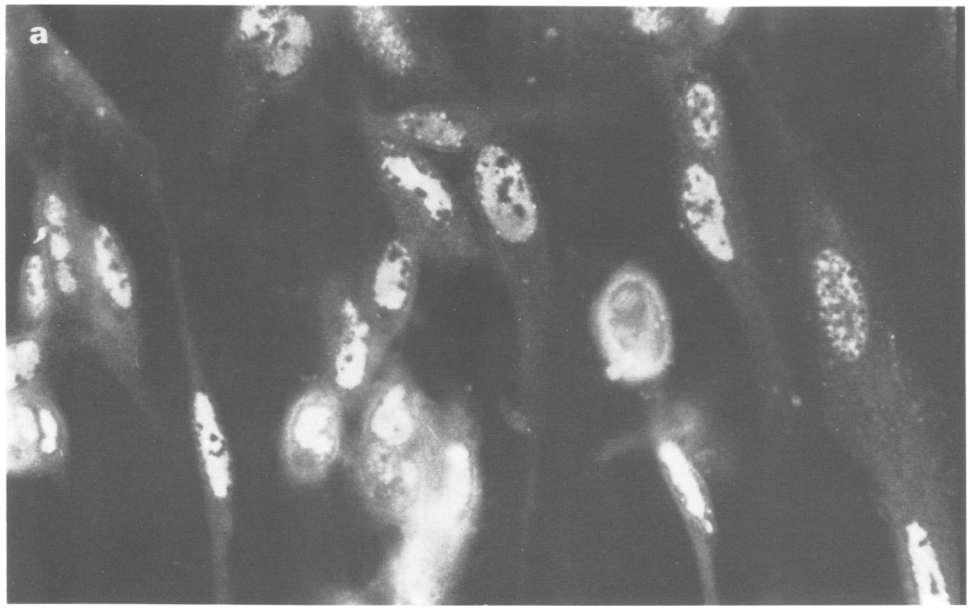

Fig. 5a

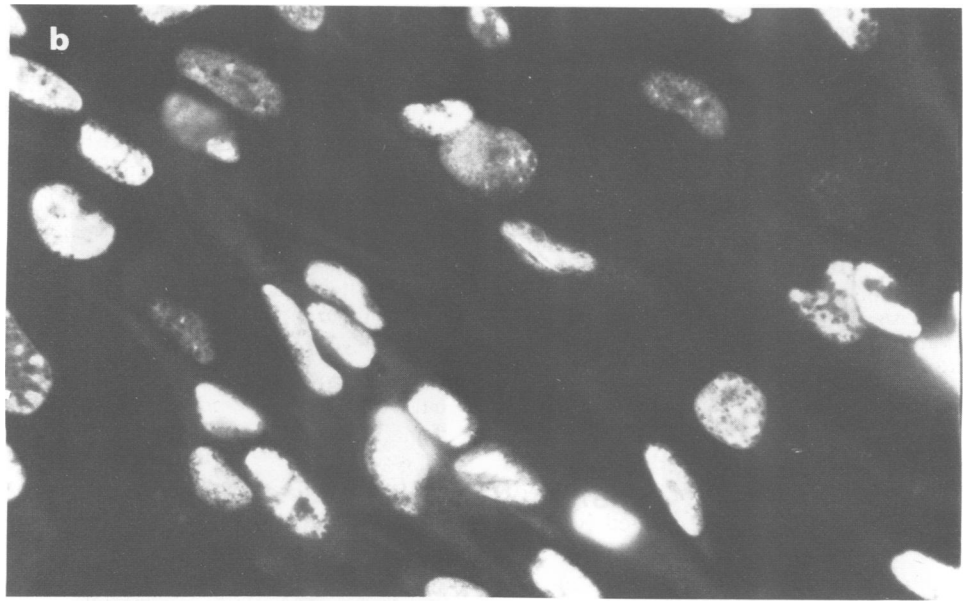

Fig. 5b

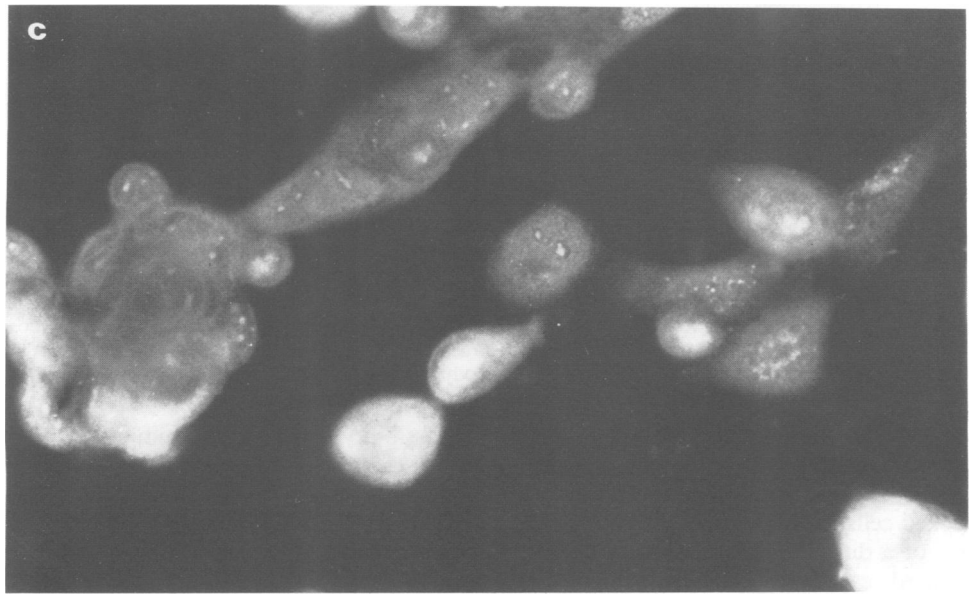

Fig. 5c

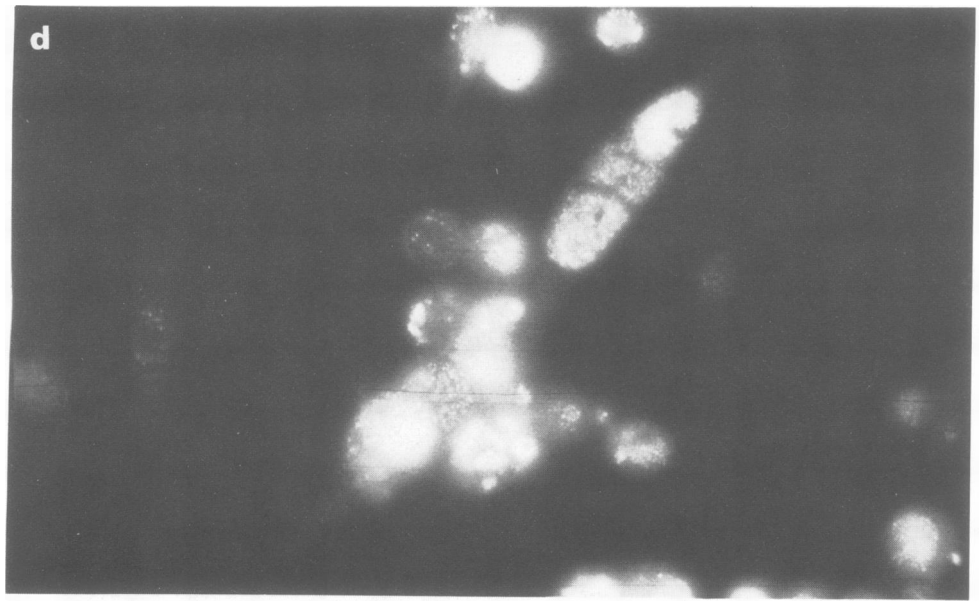

Fig. 5d

Fig. 5. Immunofluorescence with antibody TI56 on HSV 2-infected cells. Human embryo or BHK 21 cells were infected at a multiplicity of infection equal to 5, and studied by immunofluorescence: **(a)** and **(c)** human embryo with TI56, **(b)** human embryo with E9F10 **(d)** BHK 21 cells with TI56. Cells were studied at 6 h p.i. **(a)** and **(b)**, 16 h p.i. **(c)** and **(d)**. There was no fluorescence with a control antibody (not shown). Magnification 630x.

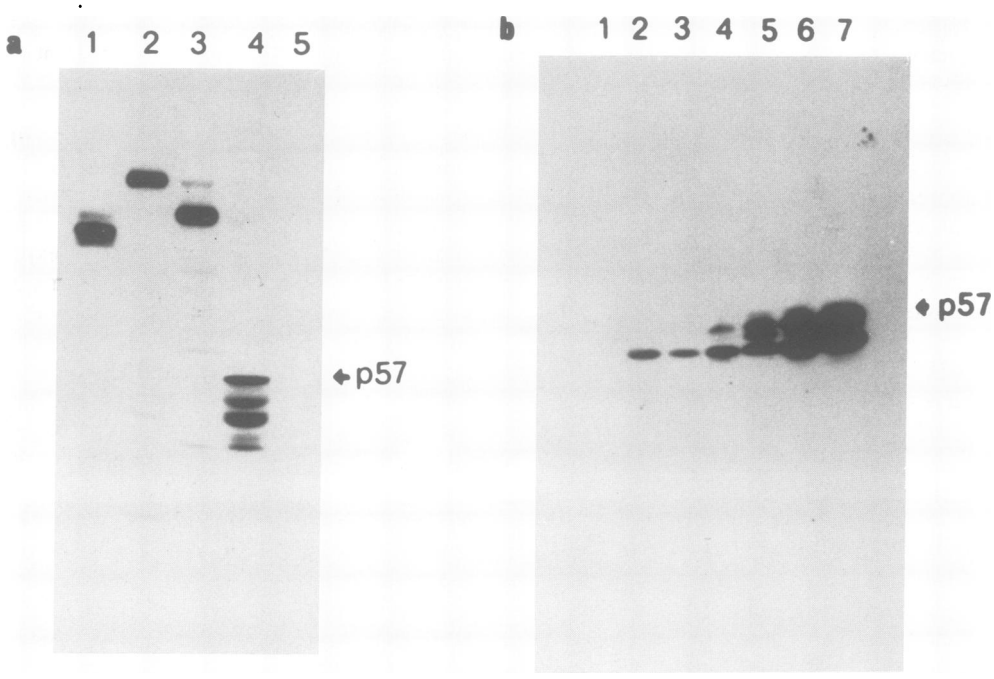

Fig. 6. Radioimmunoprecipitation and immunoblotting with antibody TI56 in HSV 2-infected cells. **(a)** Confluent cell monolayers of BHK 21 cells were infected with HSV 2 and radiolabelled as described. At 16 h p.i. they were harvested and solubilised in RIPA buffer which was used for immunoprecipitation as described. Immunoprecipitation was performed with **(1)** TG102, **(2)** TG18, **(3)**, E9F10, **(4)** TI56, **(5)** H4. This autoradiograph results from a 1-day exposure. **(b)** Confluent cell monolayers of BHK 21 cells were infected with HSV 2 and harvested at intervals by solubilising in SDS sample buffer. Samples from each time point were electrophoresed, transferred to nitrocellulose and treated with antibody TI56 as described. This autoradiograph results from a 4 h exposure to the blot, and illustrates the binding of TI56 to mock-infected cells **(track 1)**, 2 h p.i. **(track 2)**, 4.5 h p.i. **(track 3)**, 6.5 h p.i. **(track 4)**, 8 h p.i. **(track 5)**, 10 h p.i. **(track 6)**, 11.5 h p.i. **(track 7)**. There was no binding of antibody H4 to a similar blot (not shown).

associated with intensely staining structures (Figure 8a); some nuclei had a continuous rather than granular staining distribution. These data suggest that this molecule accumulates in heat-shocked cells. However, we could not rule out mere redistribution of a diffuse molecule, giving the appearance of an increased concentration. We tested this possibility by immunoprecipitation.

Exponential cultures of BHK 21 cells were radiolabelled for 30 min at 45°C, and for an additional 30 min post-heat-shock, when they were solubilised in RIPA buffer and immunoprecipitated with TI56 (Figure 9a). A control culture was also radiolabelled. Antibody TI56 immunoprecipitated

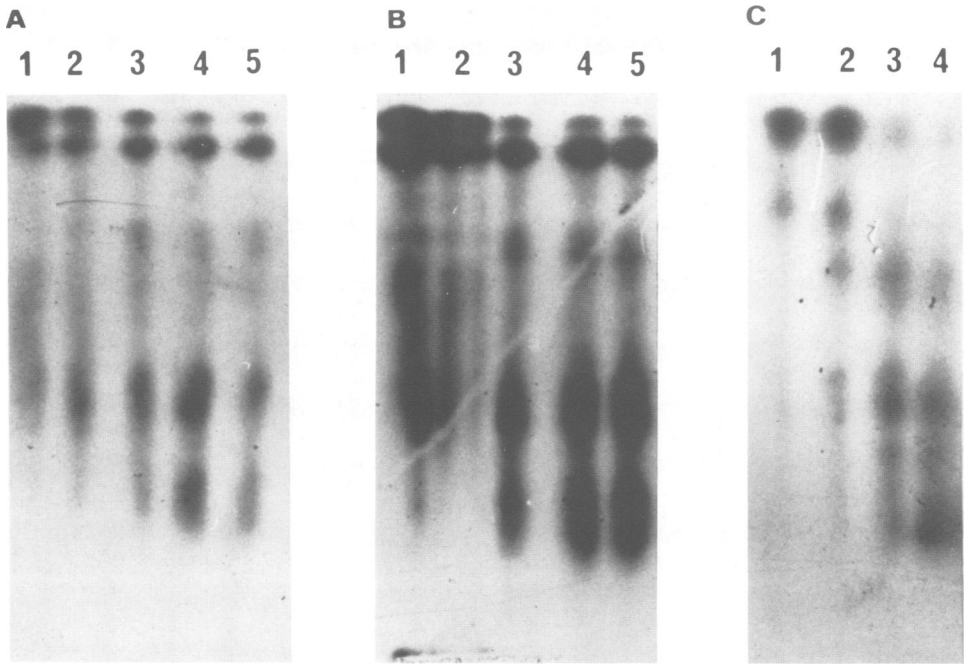

Fig. 7. Peptide mapping of p57 purified from HSV 2-infected, mock-infected confluent and heat-shocked cells. Confluent cell monolayers of BHK 21 cells were infected with HSV 2 or mock-infected and at 16 h p.i. were harvested and solubilised in RIPA buffer. Heat-shocked cells were incubated at 45°C for 30 min and similarly harvested. Immunoadsorbent affinity chromatography was performed as described, when the purified molecules were iodinated by the chloramine T method. After electrophoresis, the p57 polypeptide was located, excised, and digested with *S. aureus* V8 protease and further electrophoresed in 18% acrylamide gel. This figure shows the peptide digests for p57 from **(A)** confluent mock-infected **(B)** HSV 2-infected and **(C)** heat-shocked cells. **Tracks 1–5** represent the peptide maps after digestion with 0.0005, 0.005, 0.05, 0.5 and 5.0 μg V8 protease, respectively.

from heat-shocked cell extracts a predominant polypeptide of 57 000 mol. wt. (p57) at an increased concentration compared with that in control cultures (Figure 9a, tracks 2 and 4). Again, these results suggest that the TI56 molecule accumulates within heat-shocked cells. This was confirmed by *S. aureus* V8 protease peptide mapping using immunoadsorbent-purified p57, when the peptide digests were clearly related (Figure 7).

In support of this, BHK 21 cells were radiolabelled for 3 h in the presence of 10 μM disulfiram, a treatment known to induce the synthesis of heat-shock proteins (Levinson *et al.*, 1978). When these cells were solubilised, the TI56 antibody immunoprecipitated p57 at an increased concentration (Figure 9b, track 2) compared with the control. Taken together, these results indicate that this molecule accumulates within heat-shocked cells.

Discussion

HSV causes a rapid shut-off in host cell macromolecular synthesis (Fenwick and Walker, 1978) and during replication, gene expression is co-ordinately regulated and sequentially ordered in a cascade fashion (Honess and Roizman, 1974). We have produced monoclonal antibodies that react with infected cells, and characterised those which recognise DBPs (LaThangue and Chan, 1983). Here we report an antibody which reacts with a host cell encoded molecule that accumulates in HSV 2-infected cells.

In uninfected cells its nuclear location was growth regulated, and immunoprecipitation showed that there were small variations in mol. wt. which relate to the growth state of the cells. In exponential cells the major polypeptide was p57, whereas in quiescent cultures it was p61. Since this polypeptide is phosphorylated (data not shown), these differences could be explained by differences in phosphorylation of the same molecule (LaThangue, unpublished). However, we cannot exclude that p61 and p57 are products of different genes with a shared epitope and whose expression is growth regulated, although this possibility seems intrinsically unlikely. The consistent relationship to growth, nuclear location, and affinity for DNA suggest that this antigen has an important function within uninfected cells.

When confluent cells were infected with HSV 2 TI56-specific immunofluorescence increased with time. Several polypeptides were immunoprecipitated, ranging from 46 000 to 57 000 mol. wt., at a concentration comparable with virus polypeptides. Although there were small variations in the TI56 molecule with cell growth, the similar mol. wts. of the polypeptides in infected and uninfected cells suggests that TI56 recognises the same molecule in both cases. This was confirmed by *S. aureus* V8 protease peptide mapping.

By immunoblotting, antibody TI56 was shown to react with a 46 000 mol. wt. polypeptide at 2 h p.i., and subsequently the concentration of this and other polypeptides of greater mol. wt. increased. This is compatible with the hypothesis that the lower mol. wt. polypeptides are precursors to p57, and that it was these which were immunoprecipitated with p57 from infected cell extracts. The 46 000 mol. wt. polypeptide may accumulate first because it is the initial product in the processing pathway which is slower in HSV 2-infected cells. These changes in apparent mol. wt. may result from post-translational modification, but again we cannot exclude that they are products of different genes, perhaps comprising a family with related functions. The fact that p57, and not p61, accumulated in infected confluent cells, and that p57 accumulated within exponential cells is intriguing. It may

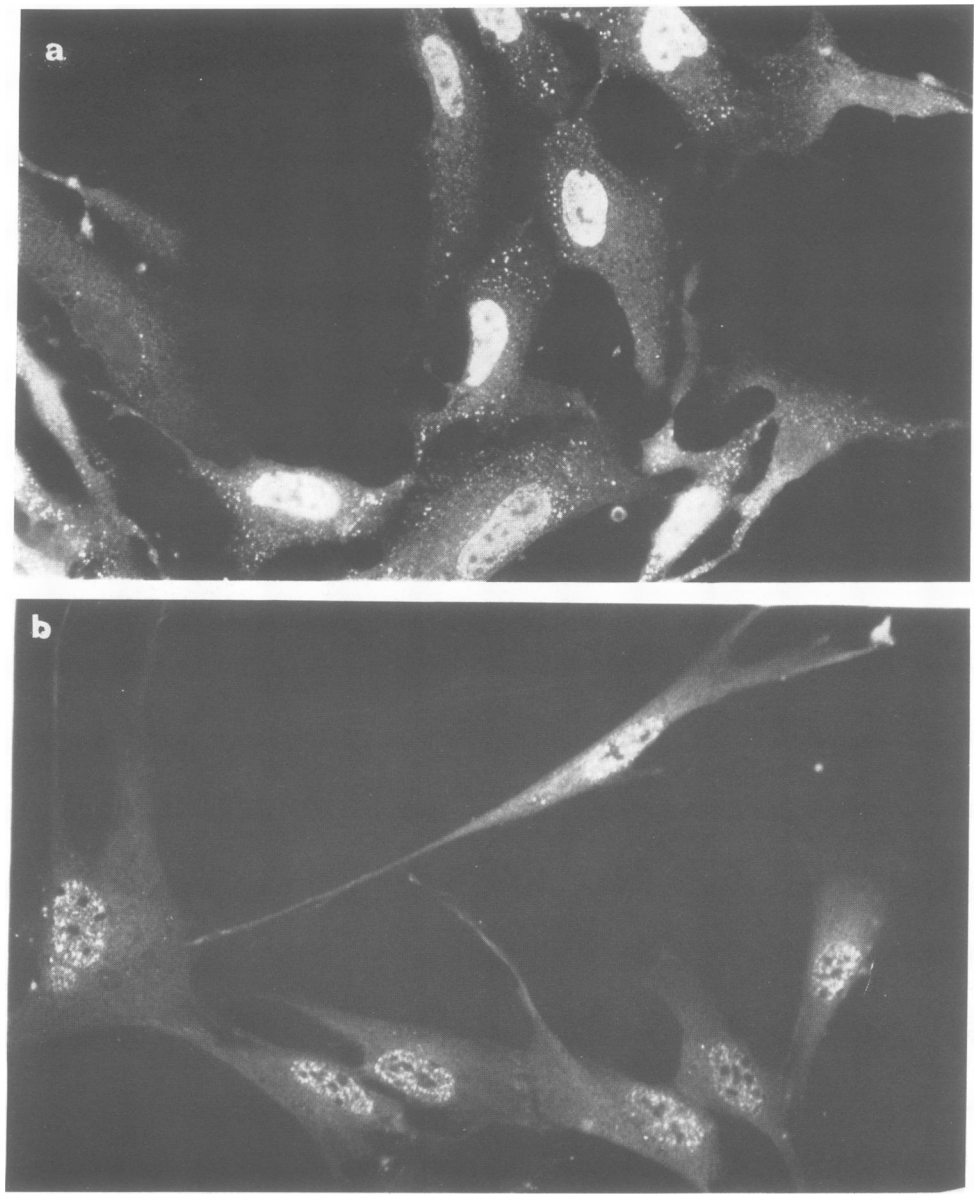

Fig. 8. Immunofluorescence with antibody TI56 on heat-shocked cells. Exponentially growing human embryó cells were incubated at 46°C for 30 min, and immunofluorescence performed with antibody TI56. **(a)** heat-shock **(b)** control non-heat-shocked culture. There was no immunofluorescence with a control antibody (not shown). Magnification 630x.

be that p57 has a similar function in both situations.

In uninfected cells, the TI56-defined molecule accumulated during heat-shock treatment and in the presence of disulfiram, which induces the synthesis of heat-shock proteins (Levinson *et al.*, 1978). By immunofluorescence, this molecule was located in the cytoplasm, and by immunoprecipitation there was an increased concentration of p57. This suggests that the TI56 molecule is not a major heat-shock protein (Kelly and Schlesinger, 1978), but rather one of the minor heat-shock proteins characterized by several workers (Buzin and Peterson, 1982; Bensaude and Morange, 1983). The fact that this gene is expressed in the 'unstressed' cell agrees with previous reports (Kelly and Schlesinger, 1982; Velazquez *et al.*, 1980; 1983; Kloetzel and Bautz, 1983; Bensaude and Morange, 1983; Buzin and Peterson, 1982) and suggests that heat-shock proteins have a general function outside the stress response.

Heat-shock proteins accumulate within some virus-infected cells (Nevins, 1982; Collins and Hightower, 1982; Khandjian and Türler, 1983). In one case this is under the control of a well-defined early gene product (Nevins, 1982), and a pseudorabies virus immediate early gene may interact with a similar cellular factor that regulates expression of heat-shock protein genes (Feldmann *et al.*, 1982). The synthesis of the major heat-shock proteins is induced by HSV 1 with temperature-sensitive mutations within the immediate early gene ICP4 (Vmw 175) although not by wild-type virus (Notarianni and Preston, 1982). Using a monoclonal antibody, we have unequivocally demonstrated that the TI56-defined molecule accumulates within wild-type HSV 2-infected cells, and that this also occurs during heat-shock; other experiments have shown that this molecule accumulates within HSV 1-infected cells, but to a lower level (LaThangue, in preparation). Therefore, it appears that some, but not all, heat-shock proteins accumulate within HSV-infected cells.

We refer to this phenomenon as accumulation, rather than

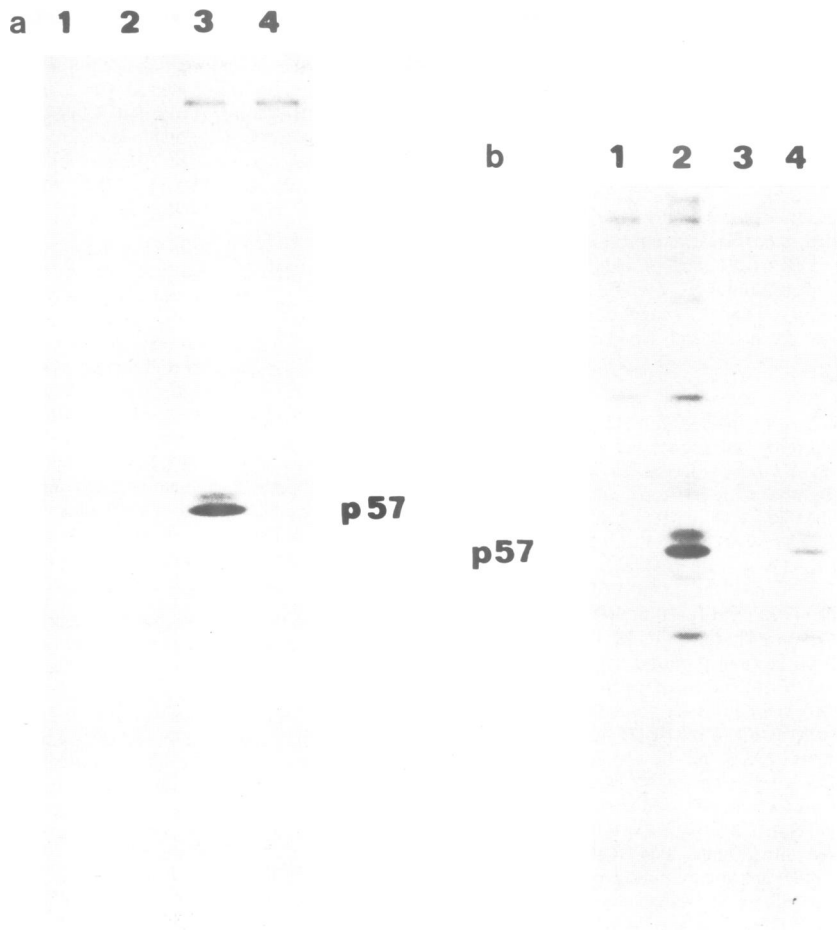

Fig. 9. Radioimmunoprecipitation from heat-shocked and disulfiram-treated cells. **(a)** Confluent cultures BHK 21 cells were radiolabelled as described at 45°C for 30 min, and for an additional 30 min at 37°C. Control cultures were radiolabelled for 1 h. Cells were solubilised and immunoprecipitated with antibody TI56 (**tracks 1** and **3**) or H4 (**tracks 2** and **4**). Immunoprecipitates from control (**tracks 1** and **2**) and heat-shocked cultures (**tracks 3** and **4**). This autoradiograph results from a 3-day exposure. **(b)** Confluent cultures of BHK 21 cells were radiolabelled as described for 4 h in the presence of 10 μM disulfiram. Control cultures were radiolabelled for 4 h. Cells were solubilised and immunoprecipitated with antibody TI56 (**tracks 2** and **4**) or H4 (**tracks 1** and **3**). Immunoprecipitates from control (**tracks 3** and **4**) and disulphirum-treated cells (**tracks 1** and **2**). This autoradiograph results from a 5-day exposure.

induction of gene activity, because the mRNA was not investigated. It is possible that this molecule has a high turnover rate in the cell, and that its accumulation following infection results from an inhibition of breakdown.

The fact that this molecule accumulates in both HSV 2 and heat-shocked cells suggests that both stimuli activate a stress response, and therefore that the TI56-defined molecule has a similar function in both situations. These results show that there are similarities, although one clear difference was in the concentrations; HSV 2-infected cells had a much greater concentration than heat-shocked cells. We have also found other differences between these two situations, such as in the polypeptides which co-immunoprecipitate with p57 and in the solubility of p57 (LaThangue, unpublished). Perhaps a given stress protein may function differently depending on the stress treatment.

A shut-off in host cell macromolecular synthesis occurs after HSV 2 infection (Hill *et al.*, 1983; Fenwick and Walker, 1978). The evidence presented here suggests that this is not complete, and that at least one cellular gene product continues to be synthesised. However, this does not appear to be a unique cellular gene, as our other monoclonal antibodies have identified two more molecules related to heat-shock proteins, which also accumulate in HSV-infected cells (LaThangue, in preparation).

At present, we can only speculate on what function this molecule has. Normally, heat-shock enhances the expression of heat-shock genes, with the repression of all other genes at both transcriptional and translational levels (Ashburner and Bonner, 1979). It is the heat-shock proteins themselves which probably mediate this effect (Peterson and Mitchell, 1981). Because of this, we suggest that the well characterised shut-off which occurs within HSV-infected cells is not a direct effect of a virus gene product, but rather an indirect effect resulting from the activated stress response. In infected cells, the virus may overcome the effects of the stress response, by having, for example, genes with features in common with heat-shock protein genes, or by encoding molecules whose activity in 'stressed' cells is not inhibited.

In conclusion, we have characterised a host cell protein, which in uninfected cells had a nuclear location which was growth regulated. In HSV 2-infected cells it accumulated to a greater concentration than that in uninfected cells. This accumulation also occurred in heat-shocked cells. We suggest that this molecule has an important function in HSV infections.

Materials and methods

Cell culture and viruses

BHK 21 and human embryo fibroblasts (Flow 5000) were grown in Eagle's medium containing 10% new born calf serum. Hybrid cell lines were grown in RPMI 1640 containing 10% foetal calf serum. HSV 2 strain 333 (Seth *et al.*, 1974) was used throughout.

Monoclonal antibodies

Hybridoma T156 was produced and cloned as previously described (Howes *et al.*, 1979). Hybridomas TG18, TG102 and TGH3 were produced from mice immunized with affinity-purified DBP (LaThangue and Chan, 1983). Hybridoma H4 was produced from mice immunized with DBP purified from the Epstein-Barr virus transformed Raji cell line (LaThangue and Clarke, unpublished). Hybridoma E9F10 was produced and cloned as previously described (Goldstein *et al.*, 1983).

Solid phase enzyme immunoassay

Confluent monolayers of BHK 21 cells were infected with HSV 2 strain 333 at a multiplicity of 1. After a 1 h adsorption, the monolayer was washed and medium added. At 16 h p.i., infected and mock-infected cells were harvested. These were washed three times in phosphate-buffered saline (PBS) and sonicated in a small volume of 20 mM Tris-HCl pH 7.5, 2 mM β-mercaptoethanol. An equal volume of 20 mM Tris-HCl pH 7.5, 2 mM β-mercaptoethanol, 3.4 M NaCl and 10 mM EDTA was then added, and extensively dialysed into 20 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM EDTA, 2 mM β-mercaptoethanol, 10% glycerol which was centrifuged at 100 000 *g* for 1 h. This supernatant was either dialysed into 20 mM Tris-HCl pH 7.5, 10% glycerol and then used as antigen for the enyzme immunoassay, or alternatively, DBP were purified from it. For this, columns of double-stranded calf thymus DNA-cellulose (Sigma, 4 mg/g cellulose) were poured in column buffer [20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 10% glycerol, 2 mM β mercaptoethanol containing 500 μg/ml bovine serum albumin (BSA)], washed with column buffer containing 2.0 M NaCl, and finally equilibrated with column buffer. The supernatant was loaded, adsorbed, and extensively washed. Bound DBP were eluted in a single step with 2.0 M NaCl. The peak was located and dialysed into 20 mM Tris-HCl pH 7.5, 10% glycerol and used as antigen in the DBP enzyme immunoassay.

For the enzyme immunoassay, supernatant (DNA-celluloce column load) was diluted to 5 μg/well, whereas the DBP peak was usually neat (usually ~2 μg/well). Diluted antigen was adsorbed onto enzyme immunoassay plate wells for 1 h at 37°C and blocked with heat-inactivated new-born calf serum. Hybridoma supernatant was added and incubated for 45 min at 37°C. After washing, diluted anti-mouse immunoglobulin conjugated to horse radish peroxidase (Miles Laboratories) was added and incubated for 45 min at 37°C. After a final wash, enzyme substrate containing O-phenyldiamine was added and incubated for 20 min at room temperature in the dark. The reaction was terminated with 1.3 N H_2SO_4. The optical density at 492 nm (OD_{492}) was recorded with a Titertek Multiscan in triplicate.

Immunofluorescence

Human embryo fibroblasts were grown on sterilised coverslips. Appropriately treated cells were washed with cold PBS, and fixed for 10 min at −20°C in methanol. Hybridoma supernatant was added and incubated for 30 min at 37°C. After washing, fluorescein-conjugated goat anti-mouse immunoglobulin (Cappel Laboratories) was added and incubation continued for 30 min at 37°C. Coverslips were then mounted in glycerol and examined using a Zeiss Ultraphot II microscope. Control monoclonal antibody H4 was used throughout.

Radiolabelling and immunoprecipitation

All radiolabelling was performed in BHK 21 cells grown in 9 cm diameter plastic tissue culture plates (Nunc, Denmark), with L-[^{35}S]methionine at 50 μCi/ml (Amersham International, sp. act. >600 Ci/mmol) in Eagle's medium containing 1/5 normal concentration of methionine. For infected cell extracts, confluent cell cultures were infected with HSV 2 at a multiplicity of infection equal to 20 p.f.u./cell and after adsorption were radiolabelled for 15 h; mock-infected cell extracts were similarly prepared. Uninfected cells were radiolabelled at three stages of growth: (i) during exponential growth when cells were radiolabelled at a density of 1 x 10^6 cell/plate; (ii) when they had just reached confluence (as determined by visual observation), ~5 x 10^6 cell/plate; (iii) when they had been confluent for 36 h (post-confluence); cells were radiolabelled for 6 h. For heat-shock treatment, cells of condition (ii) were radiolabelled for 30 min at 45°C, and for a further 30 min at 37°C. Control cultures were radiolabelled over a similar period. For disulfiram treatment, cells of condition (ii) were radiolabelled for 4 h in the presence of 10 μM disulfiram (Sigma). Cell extracts were harvested, washed, and solubilised in RIPA buffer [1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS 5 mM EDTA, 1% Aprotinin (Sigma) in PBS]. After centrifugation at 100 000 *g* for 1 h at +4°C, the extract was used for immunoprecipitation with the monoclonal antibodies. Cell extracts were immunoprecipitated with monoclonal antibody and rabbit anti-mouse immunoglobulin, and immune complexes were collected with *S. aureus* Cowan A strain. The bacteria were repeatedly washed with RIPA buffer, reduced, and electrophoresed in a 12.5% acrylamide gel cross-linked with 1.2% NN′ diallytartardiamide. Gels were processed as described (Bonner and Laskey, 1974) and then exposed to Kodak Xomat X-ray film at −70°C. A monoclonal antibody, H4, reacting with a DBP in the EBV Raji cell line was used as a control throughout.

Immunoblotting

Confluent BHK 21 cell cultures were infected with HSV 2 as described. After adsorption, cells were harvested at intervals by solubilising in SDS sample buffer. Samples from each time point were electrophoresed in a 12.5% polyacrylamide gel, after which the polypeptides were transferred into 0.4 μm nitrocellulose (Schleicher and Schüll) as described (Towbin *et al.*, 1979). The nitrocellulose was blocked by incubating it in 0.9% NaCl, 10 mM Tris-HCl pH 7.5 containing 5% BSA. Hybridoma supernatant (pH 7.5) was added and incubated for 1 h at room temperature. The nitrocellulose sheet was then extensively washed with 0.9% NaCl, 10 mM Tris-HCl pH 7.5 and the same containing 0.5% NP-40. Iodinated rabbit anti-mouse immunoglobulin was added (7.5 x 10^6 c.p.m.) diluted in Tris-saline containing 5% BSA for 1 h at room temperature. The blot was finally washed, dried, and X-ray film exposed to it. Control experiments were performed with H4 supernatant.

Immunoadsorbent affinity chromatography

Purified monoclonal antibody was coupled to Sepharose CL4B (Pharmacia Fine Chemicals) using sodium metaperiodate activation (Wilson and Nakane, 1976). The immunoadsorbent purification procedure has been described previously (Chan, 1983; LaThangue and Chan, 1983). Briefly, RIPA cell extracts were applied, adsorbed, and the column extensively washed with (i) PBS, (ii) RIPA buffer, (iii) 9 M ethanediol, 0.3 M sodium chloride, 7.5 mM sodium phosphate buffer pH 7.4, and bound molecules eluted at pH 2.0 with 9 M ethanediol, 0.3 M sodium chloride, 0.1 M citric acid. The pH was immediately neutralized, the purified molecules concentrated, and then radiolabelled with Na ^{125}I (Amersham International) by the chloramine T method.

Peptide mapping

Peptide mapping was performed essentially as described by Cleveland *et al.* (1977). Briefly, iodinated immunoadsorbent purified molecules were electrophoresed and the polypeptides located by fluorography. They were then excised and rehydrated in 125 mM Tris-HCl, pH 6.8, 0.1% SDS, and 1 mM EDTA. Mashed gel slices were placed in sample wells, overlayed with Staphylococcal V8 protease (Miles Laboratories), and digested for 1 h in the stacking gel during subsequent electrophoresis. Electrophoresis was in an 18% acrylamide gel cross-linked with 1.2% DATD.

Acknowledgements

We thank Professors M.C.Raff, R.O.Hynes and N.A.Mitchison for advice and criticism. N.L.T. was in receipt of a Science and Engineering Research Council CASE Award.

References

Ashburner,M. and Bonner,J.J. (1979) *Cell,* **17**, 241-254.
Baringer,J.R. (1974) *New Eng. J. Med.,* **291**, 818-830.
Bensaude,O. and Morange,H. (1983) *EMBO J.,* **2**, 173-177.
Bishop,J.M. (1981) *Cell,* **23**, 5-6.
Bonner,W.M. and Laskey,R.A. (1974) *Eur. J. Biochem.,* **46**, 83-88.
Buzin,C.H. and Peterson,N.S. (1982) *J. Mol. Biol.,* **158**, 181-201.
Chan,W.L. (1983) *Immunology,* **49**, 343-351.
Cleveland,D.W., Fischer,S.G., Kirshchner,M.W. and Laemmli,U.K. (1977) *J. Biol. Chem.,* **252**, 1102-1106.
Collins,P.L. and Hightower,L.E. (1982) *J. Virol.,* **44**, 703-707.
Feldman,L.T., Imperiale,M.J. and Nevins,J.R. (1982) *Proc. Natl. Acad. Sci. USA,* **79**, 4952-4956.
Fenwick,M.L. and Walker,M.J. (1978) *J. Gen. Virol.,* **41**, 37-51.
Galloway,D.A. and McDougall,J.K. (1983) *Nature,* **302**, 21-24.
Goldstein,L.C., Corey,L., McDougall,J.K., Tolentino,E. and Nowinski, R.C. (1983) *J. Infect. Dis.,* **147**, 829-837.
Haarr,L. and Marsden,H.S. (1981) *J. Gen. Virol.,* **52**, 77-92.
Hayward,W.S., Neal,B.G. and Astrin,S.M. (1981) *Nature,* **290**, 475-480.
Hill,T.M., Sinden,R.R. and Sadler,J.R. (1983) *J. Virol.,* **45**, 241-250.
Honess,R.W. and Roizman,B. (1974) *J. Virol.,* **14**, 8-19.
Howes,E.L., Clark,E.A., Smith,E. and Mitchison,N.A. (1979) *J. Gen. Virol.,* **44**, 81-87.
Kelly,P. and Schlesinger,M. (1978) *Cell,* **15**, 1278-1286.

Kelly,P. and Schlesinger,M. (1982) *Mol. Cell. Biol.*, **2**, 267-274.
Khandjian,E.W. and Türler,H. (1983) *Mol. Cell. Biol.*, **3**, 1-8.
Kloetzel,P.M. and Bautz,E.K. (1983) *EMBO J.*, **2**, 705-710.
Lane,D.P. and Crawford,L.V. (1979) *Nature*, **278**, 261-263.
LaThangue,N.B. and Chan,W.L. (1983) *Arch. Virol.*, in press.
Levinson,W., Mikelens,P., Opperham,H. and Jackson,J. (1978) *Biochim. Biophys. Acta*, **519**, 65-75.
Luka,J., Jarvall,H. and Klein,G. (1980) *J. Virol.*, **35**, 592-602.
Nevins,J.R. (1982) *Cell*, **29**, 913-919.
Nishioka,Y. and Silverstein,S. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 2370-2374.
Notarianni,E.L. and Preston,C.M. (1982) *Virology*, **123**, 113-122.
Peterson,N.S. and Mitchell,H.K. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 1708-1711.
Read,G.S. and Frenkel,N. (1983) *J. Virol.*, **46**, 498-512.
Sarnow,P., Ye Shih Ho, Williams,J. and Levine,A.J. (1982) *Cell*, **28**, 387-394.
Seth,P., Rawls,W.E., Duff,R., Rapp,F. and Melnick,J.L. (1974) *Intervirology*, **3**, 1-14.
Sydiskis,R.J. and Roizman,B. (1966) *Science (Wash.)*, **153**, 76-78.
Towbin,H., Staehelin,T. and Gordon,J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4350-4354.
Wilson,M.B. and Nakane,P.K. (1976) *J. Immunol. Methods*, **12**, 171-181.
Velazquez,J.M., Di Domenico,B.J. and Lindquist,S. (1980) *Cell*, **20**, 679-689.
Velazquez,J.M., Sonada,S., Bugaisky,G.E. and Lindquist,S. (1983) *J. Cell Biol.*, **96**, 286-290.

Received on 16 August 1983; revised on 10 November 1983