The EMBO Journal vol.4 no.12 pp.3223-3228, 1985

Cellular proteins expressed in herpes simplex virus transformed cells also accumulate on herpes simplex virus infection

Joan C.M.Macnab[1], Alexander Orr and Nicholas B.La Thangue[2,3]

[1]MRC Institute of Virology, University of Glasgow, Church Street, Glasgow G11 5JR, and [2]Imperial Cancer Research Fund, Tumour Immunology Unit, University College London, Gower Street, London WC1E 6BT, UK

[3]Present address: Cancer Research Campaign, Eukaryotic Genetics Research Group, Department of Biochemistry, Imperial College, London SW7 2AZ, UK

Communicated by J.Subak-Sharpe

The cell proteins expressed in rat embryo cells transformed by herpes simplex virus (HSV) have been analysed by immunoprecipitation assays to determine those polypeptides which can be identified by immunoprecipitation with the sera of tumour-bearing animals and also with antisera to herpes simplex infected cells. Cell polypeptides commonly recognised by both these sera have been further characterised using a monoclonal antibody directed against a cellular polypeptide which accumulates on HSV-2 lytic infection. This monoclonal antibody recognises in HSV-transformed cells polypeptides of mol. wts. 90 000, 40 000 and 32 000. Further studies show that the accumulation of these polypeptides in HSV-transformed cells is not HSV specific but is a common feature of transformation or of cells which have been immortalised. We suggest that cellular polypeptides accumulating as a result of HSV infection may be of importance in the initiation of transformation by HSV, i.e., at the level of immortalisation of cells.

Key words: transforming proteins/HSV transformation/cell regulatory proteins

Introduction

Infection with herpes simplex virus has been associated for over two decades with cervical neoplastic disease (Naib *et al.*, 1966; Adam *et al.*, 1973). Despite a recent comprehensive study (Vonka *et al.*, 1984) suggesting no associations between the virus and cervical disease, a wide body of literature has associated the virus with at least some stage in the progression of cervical neoplasia (zur Hausen, 1982) even though human papilloma virus genomes have now been detected frequently in both pre-invasive (Gissmann *et al.*, 1983) and invasive cervical disease (Dürst *et al.*, 1983; Boshart *et al.*, 1984). In addition, HSV DNA sequences have been implicated at least in the initiation of HSV-2 cellular transformation *in vitro* by Reyes *et al.* (1979), Cameron (Macnab and McDougall, 1980), Galloway (Macnab and McDougall, 1980), Jariwalla *et al.* (1980), Galloway and McDougall (1981) and Cameron *et al.* (1985), but no HSV-coded transforming protein has been identified (Galloway and McDougall, 1983; Cameron *et al.*, 1985). HSV DNA sequences are present in at least 10% of genital tumours (Galloway and McDougall, 1983; Park *et al.*, 1983; Macnab *et al.*, 1985).

A role for HSV in the initiation of transformation has been shown by two animal model systems. In the first, both u.v. light and the tumour promotor, TPA, are cofactors which together with HSV-1 induce on the mouse lip papillomas which can progress to carcinomas (Burns and Murray, 1981). In the second, the application of HSV-2 to the mouse cervix induces cervical dysplastic changes in which the lesions progress to carcinomas (Wentz *et al.*, 1981). Similar treatment of mice that had been previously immunised with HSV-2 prevents the progression to carcinomas (Wentz *et al.*, 1983).

Here we present evidence which shows that cellular polypeptides expressed at high levels in rat cells transformed by HSV and by other DNA or RNA viruses and in immortalised cell lines are similar to cellular polypeptides which accumulate on HSV infection. However, these same polypeptides are not detectable in primary or secondary rat cell cultures studied as controls. This leads us to propose that the induction of cellular genes may be a mechanism by which HSV can initiate cellular transformation.

Results

Polypeptides visualised on 6.5-15% polyacrylamide gels

Initially we compared on linear 6.5-15% polyacrylamide gels (data not shown) the polypeptides extracted from secondary cultures of Hooded Lister rat embryo cells and from such cells transformed by the *Bgl*II n fragment of HSV-2 strain HG52 (Cameron *et al.*, 1985). The only difference consistently observed was one seen in Figure 1 in the region of mol. wt. 40 000 where the transformed or tumour cells Bn3, Bn5 and Bn5T (B3, B5 and B5T Figure 1, tracks 12, 1, 4) show one intense band at 40 000 whereas the control rat embryo cells (RE Figure 1, track 9) show two much less intense bands in this mol. wt. region. This difference (data not shown) was also observed in cells transformed by sheared DNA or by *ts* mutants at NPT i.e. cell lines REα RE2A, REAsyn$^+$C, RE1 and RE7 (see Materials and methods) or by virus at supraoptimal temperature (see Materials and methods, Cameron *et al.*, 1985).

Immunoprecipitation studies with antisera from tumour-bearing rats

One of the cell lines examined, Bn5T, was derived by culture of a tumour induced by Bn5 cells. We used the sera from Bn5T tumour-bearing rats i.e., tumour-bearing sera (TBS) to ask if we could immunoprecipitate polypeptides from HSV-transformed cells which were not precipitable from secondary rat embryo control cells.

Several polypeptides were precipitated from transformed and tumour cells (B5 and B5T respectively, Figure 2, tracks 5, 1) which were not immunoprecipitated from control secondary cells (RE Figure 2, track 6). The polypeptide immunoprecipitated from transformed cells at greatest levels was seen migrating at mol. wt. 90 000 (Figure 2, tracks 1, 5). Several others, including those of 60 000, 40 000 and 21 000, were also seen. We tested in a similar manner the other HSV-transformed cells (see Materials and methods) which we had run on linear gels to examine the polypeptide profiles. Similar results were obtained for polypeptides immunoprecipitated from these HSV-transformed cells with TBS (data not shown).

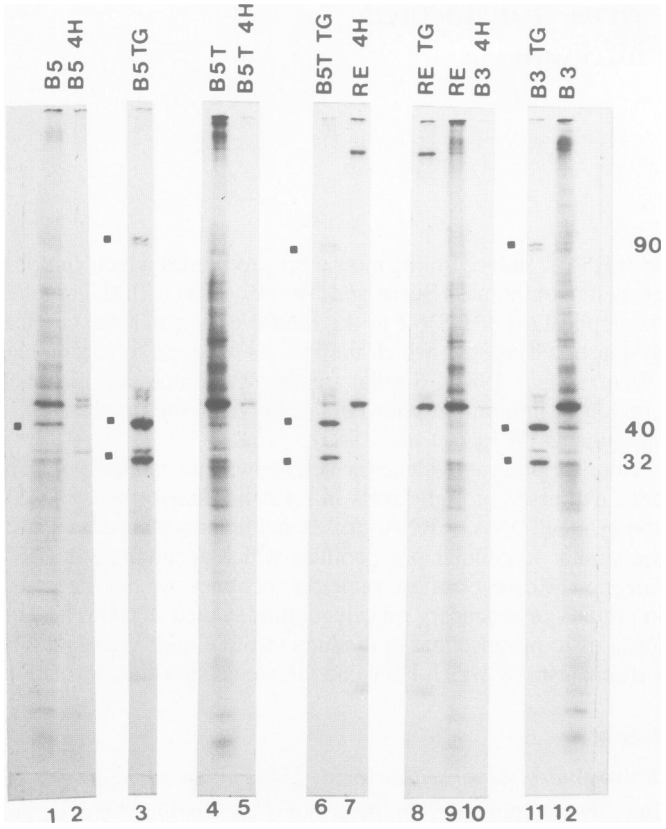

Fig. 1. Autoradiograph of 6.5 – 15% discontinuous gradient gel containing electrophoretically separated [^{35}S]methionine-labelled polypeptides from control rat embryo (RE) cells **track 9**, B5 cells (derived by transformation of RE cells with the cloned *Bgl*II n fragment of HSV-2, strain HG52) **track 1**, B5T cells (tumour cells induced by inoculation of B5 cells) **track 4** and B3 cells (a separate clone induced in the same way as B5 cells) **track 12**. Immunoprecipitation with TG7A monoclonal antibody (called in this Figure TG) is shown for RE cells **track 8** (RETG), B5 cells **track 3** (B5TG), B5T cells **track 6** (B5TTG) and B3 cells **track 11** (B3TG) and controls with the 4H monoclonal antibody are shown for RE cells **track 7** (RE4H), B5 cells **track 2** (B54H), B5T cells **track 5** (B5T4H) and B3 cells **track 10** (B34H). The 4H monoclonal antibody control defines an unrelated polypeptide. Specific polypeptides labelled 90 000, 40 000 and 32 000 are immunoprecipitated from B5 cells (**track 3**) B5T cells (**track 6**) and B3 cells (**track 11**) with the TG7A monoclonal antibody (called TG in this Figure). The polypeptides of 90 000, 40 000 and 32 000 are indicated by filled-in black squares to the left of **tracks 1, 3, 4, 6, 11** and **12**. The mol. wt. is indicated to the right of the gel.

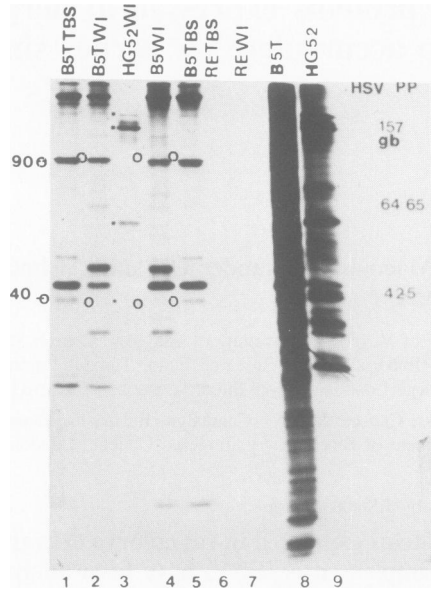

Fig. 2. Autoradiograph of 6.5 – 15% discontinuous gradient polyacrylamide gel containing electrophoretically separated [^{35}S]methionine-labelled polypeptides from the following cells: control rat embryo (RE) cells (secondary and tertiary), immunoprecipitated with sera from tumour-bearing rats (TBS) **track 6** (RETBS) and with an antiserum made against rat embryo cells infected with HSV-2 strain HG52 for 7.5 h (WI) **track 7** (REWI). B5 cells (Bn5 cells derived by transformation of RE cells with *Bgl*II n cloned fragment of HSV-2 strain HG52) **tracks 4** and **5**, immunoprecipitated with WI antiserum (**track 4**) (B5WI) and TBS antiserum (**track 5**) (B5TBS). B5T cells (tumour cells induced by inoculation of B5 cells) immunoprecipitated with TBS, (**track 1**) (B5TTBS) and immunoprecipitated with WI, (**track 2**) (B5TWI). **Track 8** is the polypeptide profile of B5T cells. **Track 9** is the polypeptide profile of RE cells infected with HSV-2 strain HG52 for 7.5 h and **track 3** is the same HSV-2 infection as **track 9** but shows the polypeptides immunoprecipitated with the WI antiserum. Specific cellular polypeptides are precipitated from B5T (**track 1**) (B5TTBS) and B5 (**track 5**) (B5TBS) cells with TBS antiserum and from B5T (**track 2**) (B5TWI) and B5 (**track 4**) (B5WI) with WI antiserum. **Track 3** was immunoprecipitated using 1×10^5 c.p.m. of [^{35}S]methionine-labelled polypeptides whereas **tracks 1, 2, 4, 5, 6** and **7** employed 4×10^6 c.p.m. of ^{35}S-labelled proteins. This result shows that in infected cells the HSV-specific polypeptides are present in much greater amounts than the cell proteins upregulated on HSV infection since the cellular 40 000 polypeptide is not seen in **track 3** in which only 1×10^5 c.p.m. of ^{35}S-labelled protein are precipitated. The polypeptides of 90 000 and 40 000 are indicated by open circles to the left of **tracks 1, 2, 4** and **5**. The HSV polypeptides are indicated by filled-in squares to the left of **track 3**. The HSV mol. wts. are indicated to the right of the gel and the cell polypeptide mol. wts. to the left of the gel.

Immunoprecipitation studies with antisera raised against HSV-2 infected rat cells

To establish whether any of the polypeptides thus immunoprecipitated by TBS from HSV-transformed cells were polypeptides which also accumulated during HSV lytic infection of cells we made antisera (WI) to HSV-infected rat cells. Immunoprecipitation with this polyclonal anti HSV-2 antiserum (WI) of HSV-transformed cells detects polypeptides migrating with mol. wts. 90 000 and 40 000 in HSV-transformed cells (Figure 2, tracks 2,4) but does not do so in control primary cells (Figure 2, track 7). Several other HSV-infected rat cell polyclonal antisera gave similar results (data not shown).

We determined the specificity of the HSV antisera to HSV-specified polypeptides by carrying out immunoprecipitation assays with HSV-infected rat embryo cells (data not shown) and identifying 20 – 30 polypeptides which either co-migrated with known standard polypeptides (Marsden *et al.*, 1978) or co-migrated with purified virion polypeptides separated on similar gels (data not shown). These results confirmed that the antisera to HSV-2 infected cells also contained antibodies to both HSV antigens and cellular polypeptides.

Immunoprecipitation studies with monoclonal antibodies directed against cellular polypeptides

To study the nature of the proteins induced in B5 and B5T cells further, several monoclonal antibodies known to define cellular proteins were screened by immunoprecipitation tests.

One cell monoclonal antibody, TG7A generated by La Thangue (in preparation), immunoprecipitated polypeptides of mol. wt. 90 000, 40 000 and 32 000 (Figure 1, tracks 3, 6 and 11, i.e. B5TG, B5TTG, B3TG) (i) from cells (B3 and B5) transformed by the *Bgl*II n fragment of HSV-2 strain HG52, (ii) from tumours (B5T) induced by *Bgl*II n transformed cell lines (B3 and B5) and

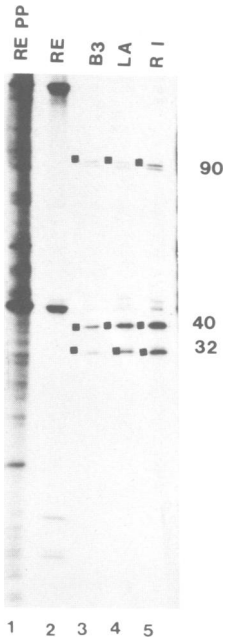

Fig. 3. Autoradiograph of 6.5−15% discontinuous gradient gel containing electrophoretically separated [^{35}S]methionine-labelled polypeptides from control rat embryo (RE) cells (**track 2**), Bn3 (B3) cells (**track 3**); Rat-1 (R1) cells transformed by LA24 *ts* mutant of RSV-labelled LA (**track 4**) and Rat-1 cells labelled R1 (**track 5**) all immunoprecipitated with the TG7A monoclonal antibody. **Track 1** shows the total proteins extracted from RE cells. Specific polypeptides labelled 90 000, 40 000 and 32 000 are immunoprecipitated from B3 (**track 3**), LA (**track 4**) and R1 (**track 5**) cells by the TG7A monoclonal antibody. The 90 000, 40 000 and 32 000 are indicated by filled-in black squares to the left of **tracks 3**−5. The mol. wt. is indicated to the right of the gel.

(iii) from other independent cell lines (data not shown) transformed by HSV *ts* mutants at non-permissive temperature (NPT) by sheared virus DNA or by virus at supraoptimal temperature (see Materials and methods). However, these polypeptide species were not detected by immunoprecipitation with TG7A from primary, secondary or tertiary rat embryo cells. Labelling of the transformed cells with [^{35}S]methionine at different stages of growth did not suggest that the TG7A monoclonal antibody detects polypeptides by immunoprecipitation assays which are cell-cycle dependent in HSV-transformed cells.

Polypeptides recognised by the TG7A monoclonal antibody are a general feature of transformed cells

Since the TG7A monoclonal antibody recognised polypeptides of 90 000, 40 000 and 32 000 in all of the HSV-transformed cell lines studied and not in primary or secondary rat cells, we tested the possibility that the expression of these polypeptides might be a general characteristic of the transformed state and not an event specific for cells transformed by HSV. Rat embryo cells transformed by Adeno 12 (Ad12), which retained Ad12 DNA sequences (Paraskeva *et al.*, 1982) or similar cells in which the Ad12 DNA sequences were no longer detectable by Southern analysis (data not shown) and rat cells transformed by Rous sarcoma virus (RSV) (Steimer and Klagsbrun, 1981) (Figure 3, track 4 LA) showed similar levels of 90 000, 40 000 and 32 000 polypeptides immunoprecipitated by the TG7A monoclonal antibody. This result indicates that expression of the TG7A defined polypeptides is a general characteristic of cellular transformation and is not HSV-transformation specific. Of course, the results obtained with the WI antiserum (made against rat embryo cells infected with HSV-2 strain HG52) and with monoclonal antibody TG7A show that these cellular polypeptides can also be induced by HSV infection (Figure 2, tracks 2 and 4).

The TG7A monoclonal antibody recognises polypeptides detectable in immortalised rat embryo cells

All the HSV-transformed cells were derived by transformation of primary, secondary or tertiary cultures. However, the parent rat cell transformed by RSV was the immortalised Rat-1 cell line (Prasad *et al.*, 1976). Several immortalised cell lines were then examined to test the possibility that conversion of a primary cell to an established cell line i.e., immortalisation might influence the expression of the 90 000, 40 000 and 32 000 polypeptides. Rat-1 (Figure 3, track 5, R1) indicating that these polypeptides are upregulated by conversion of a primary, secondary or tertiary cell to an immortalised cell. The finding that immunoprecipitation from 10 distinct different rat embryo cell preparations studied as secondary or tertiary cells were all negative confirms this conclusion.

Characterisation of the cellular polypeptides recognised by the tumour-bearing sera and by the TG7A monoclonal antibody by Staphylococcus V8 protease digestion

Finally we have carried out *Staphylococcus aureus* V8 protease digestion of the TG7A-defined cellular polypeptides synthesised in HSV-transformed rat cells and in immortalised rat cell lines and shown that the sizes of the proteolytic fragments are similar whether identified by TG7A or TBS (Figure 4a). This finding strongly suggests that the TG7A-defined polypeptides in HSV-transformed cells are also present in immortalised cells.

The region of 32 000−40 000 mol. wt. possibly represents a family of proteins recognised by the TG7A and tumour-bearing sera

High resolution gels of the polypeptides immunoprecipitated by the sera of tumour-bearing rats (TBS) and those similarly immunoprecipitated by the TG7A monoclonal antibody (Figure 4b, Tracks 4, 5, 6 and 7) showed that the 90 000 polypeptides comigrate whether they are immunoprecipitated with the TBS or the TG7A monoclonal antibody. This finding was further confirmed by comparison of *S. aureus* V8 protease digestion of the 90 000 polypeptides immunoprecipitated by TG7A and TBS in which no differences are distinguishable (data not shown). However, the polypeptide of 40 000 precipitated by the monoclonal antibody TG7A may have a slightly larger mol. wt. than the similar polypeptide immunoprecipitated by TBS (Figure 4b). This was further confirmed by *S. aureus* V8 protease digestion of the polypeptides immunoprecipitated by TBS and TG7A in the region of 30 000−40 000 (data not shown). The polypeptides of ~40 000 and 32 000 immunoprecipitated by TBS and the TG7A monoclonal antibody, respectively, were found to be similar but not absolutely identical. We interpret these results to suggest that the major antigenic component of the transformed cell eliciting an antibody response detectable by TBS and also recognised by the TG7A monoclonal antibody is the 90 000 polypeptide which is probably mainly expressed on the cell surface of the transformed and subsequent tumour cells and produces a strong immunogenic response as the tumour grows. We cannot, however, at this stage rule out the possiblity that post-translational modification may account for the differences seen in mol. wts. of polypeptides seen in the 32 000−40 000 mol. wt. region. The result also suggests that the polypeptides of mol. wts. between 32 000 and 40 000 with similar peptide maps may be related to each other.

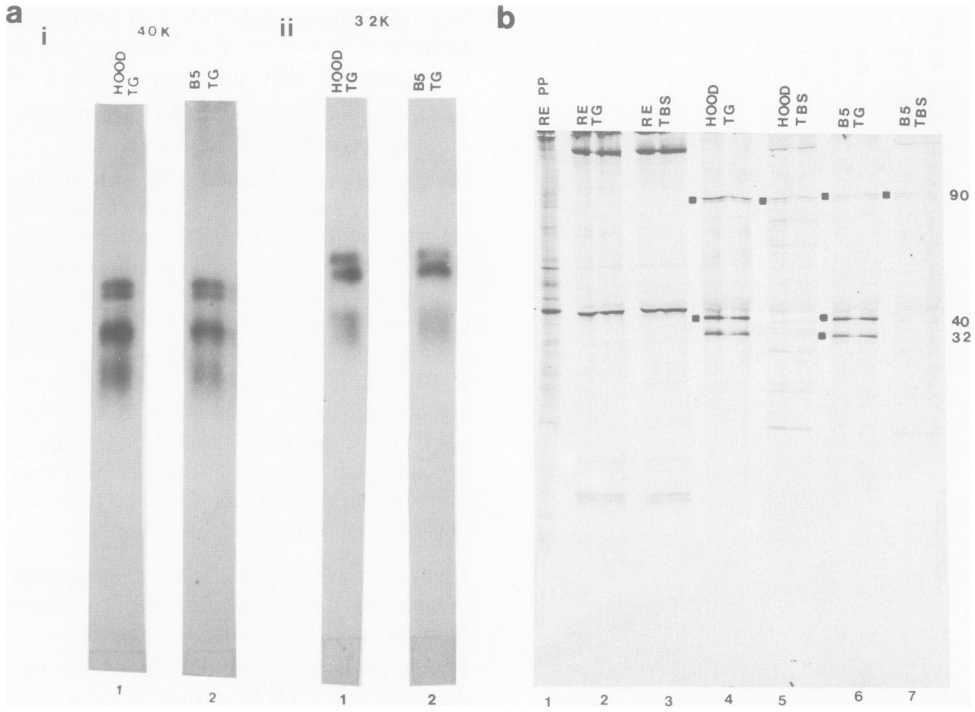

Fig. 4. (**a**) Autoradiograph of 18% polyacrylamide gel containing electrophoretically separated [^{35}S]metionine-labelled peptides which have been digested to completion by *S. aureus* V8 protease (10 μg per digest) in the stacking gel according to the protocol of Cleveland *et al.* (1977) with the modification that the original gel used to separate the polypeptides was dried, autoradiographed and the excised bands rehydrated in buffer prior to subsequent protease digestion. (**i**) Peptides separated from the 40 000 polypeptide of Hood cells (**track 1**) and B5 cells (**track 2**) immunoprecipitated by the TG7A monoclonal antibody. No differences were detected between these peptides of immortalised cells (Hood, **track 1**) and HSV-transformed cells (B5 **track 2**). (**ii**) Peptides separated from the 32 000 polypeptide of Hood cells (**track 1**) and B5 cells (**track 2**) immunoprecipitated by the TG7A monoclonal antibody. No differences were detected between the peptides of immortalised cells (Hood, **track 1**) and HSV-transformed cells (B5 **track 2**). (**b**) Autoradiograph of 6.5 – 15% discontinuous polyacrylamide gel containing electrophoretically separated [^{35}S]methionine-labelled polypeptides from control rat embryo (RE), Hood and Bn5 (B5) cells which have been electrophoresed in tracks of double the usual width and run to separate and compare the 40 000 and 32 000 components of Hood and B5 cells immunoprecipitated by TG7A monoclonal antibody (Hood TG **track 4** and B5 TG **track 6**) and TBS (Hood TBS **track 5** and B5 TBS **track 7**). The differences seen between TBS and TG7A immunoprecipitation may represent either different polypeptides in this region or a modification of the polypeptides recognised by the two immune probes. The 90 000 polypeptide clearly seen as a doublet looks similar for Hood cells (**tracks 4** and **5**) and B5 cells (**tracks 6** and **7**) whether immunoprecipitated by the TG7A monoclonal antibody (Hood **track 4**, B5 **track 6**) or the TBS (Hood **track 5**, B5 **track 7**). **Track 1** shows the polypeptide profile of RE cells while **tracks 2** and **3** show RE cells precipitated with TG7A (**track 2**, RETG) and TBS (**track 3**, RETBS). No specific bands similar to those detected by the TG7A monoclonal antibody are seen by precipitation of RE cells with either of these antisera. Other polypeptides which are changed in transformed or immortalised cells are seen in these gels but are not the subject of this study. The polypeptides of 90 000, 40 000 and 32 000 are indicated by filled-in black squares to the left of tracks 4 – 7. The mol. wt. is indicated to the right of the gel.

Discussion

A number of polypeptides were detected in HSV-transformed and tumour cells by immunoprecipitation studies using either the sera of tumour-bearing rats or antisera raised against HSV-2 infected rat embryo cells. These polypeptides were not detected in control rat embryo primary or secondary cells. This finding indicates that a number of cellular polypeptides which are upregulated on HSV infection and which produce an antibody response are similar to cellular polypeptides detectable by similar assays using sera from tumour-bearing rats (Figure 2). These cell polypeptides are either expressed in greater amounts or presented in a different manner in tumour cells than in normal cells.

One monoclonal antibody, TG7A, which was isolated by La Thangue (La Thangue and Latchman, in preparation) and which recognises cell polypeptides of mol wts. 90 000, 40 000 and 32 000 which accumulate upon HSV infection was used in this study. This monoclonal antibody was able to immunoprecipitate [^{35}S]methionine-labelled polypeptides of mol wts. 90 000, 40 000 and 32 000 from HSV-transformed or tumour cell lines. These polypeptides were not similarly immunoprecipitable from control rat embryo primary or secondary cells. Immunoblot studies also showed these same polypeptides to accumulate in both HSV-infected and transformed cells (La Thangue, personal communication). The fact that all the HSV-transformed cells tested showed this accumulation of cellular polypeptides led us to investigate whether it was transformation-specific rather than HSV-specific. The rat cells used in the Adeno 12 transformation studies were derived from an inbred Hooded Lister stock similar to the one we use in Glasgow. Results from Adeno transformation studies suggested these polypeptides were cell-transformation specific as distinct from HSV-transformation specific. Their presence in the continuous Hooded Lister cell lines established in Glasgow and also in the Rat-1 cell line derived from Fischer rats (Figure 3) suggested that the polypeptides recognised by the TG7A monoclonal antibody define the difference between a primary, secondary and tertiary rat embryo cell and a rat cell which has been immortalised. The upregulation of these polypeptides may be a key event in the conversion of cells with a finite lifespan to cells capable of indefinite growth.

Peptide analysis (Figure 4a) shows that the polypeptides of mol. wts. 40 000 and 32 000 are not different by *S. aureus* V8 protease digestion whether they are isolated from the Bn5T cells or immortalised Hood cells. Similar results were obtained for

the 90 000 mol. wt. polypeptide (data not shown).

This result confirms that the TG7A monoclonal antibody identifies a similar polypeptide complex in HSV-transformed rat cells to that which it recognises in immortalised rat cells. At mol. wt. 90 000 a similar polypeptide can be seen (Figure 4b) from peptide mapping after immunoprecipitation with the sera of tumour-bearing rats. However, at mol. wts. 32 000 and 40 000 *S. aureus* V8 protease digestion yields peptide patterns of polypeptides precipitated by tumour-bearing sera which are not identical with but are very similar to those precipitated by TG7A (data not shown). These results suggest a family of proteins identifiable in this 32 000 – 40 000 mol. wt. area. We cannot, however, rule out the possibility of processing products being identified.

Maintenance of the oncogenic phenotype in HSV-transformed rat embryo cells is not dependent on the retention of HSV DNA sequences coding for an HSV-coded transforming protein (Cameron *et al.*, 1985).

Other mechanisms by which cellular genes may be upregulated or altered in expression are by transactivation (Everett and Dunlop, 1984) of cellular promoters by HSV gene products or, as recently reported (Walker *et al.*, 1985), by the introduction of non-sequence specific double-stranded DNA. In addition, hypomethylation, seen on HSV infection, may alter cellular transcription patterns permanently and we are currently studying the effects of such hypomethylation on transcriptional control in HSV-transformed cells (R.L.P.Adams, L.Clark and J.C.M.Macnab, in preparation).

Analysis of the sequence encoded by the *Bgl*II n fragment of HSV-2 which initiates transformation (Galloway and McDougall, 1983; McLauchlan and Clements, 1983; Macnab *et al.*, 1985) does not suggest that a strong viral promoter is involved in promotional insertion as a mechanism of *Bgl*II n transformation.

In addition, the series of established cell lines examined by us has not seen either virus or transfected DNA sequences. Thus, the control of the upregulation of the host cell polypeptides recognised by the TG7A monoclonal antibody has not at this stage been shown to be a sequence-specific event.

It has been shown that phosphorylation of the 90 000 and 45 000 heat shock proteins is a response to the introduction of non-sequence specific double-stranded DNA into cells (Walker *et al.*,1985). Although the process of cell immortalisation could conceivably involve the upregulation of heat shock or stress proteins it is unlikely from mobility that the 45 000 mol. wt. polypeptide described by Walker *et al.* (1985) is the same as the 40 000 mol. wt. polypeptide described by us. However, the 90 000 mol. wt. heat shock protein described by Walker *et al.* (1985) may be similar or related to that which we described. We could not immunoprecipitate 90 000, 40 000 and 32 000 mol wt. polypeptides from heat-shocked primary rat embryo cells with the TG7A monoclonal antibody similar to those immunoprecipitated from transformed cells. This may merely reflect that such polypeptides are more abundant, more complex or presented differently in rat transformed cells. Notarianni and Preston (1982) have shown the induction of heat shock proteins including those of 90 000 and 40 000 mol. wt. in chick embryo cells by a mutant with a defect in the immediate early 175 000 mol. wt. polypeptide, HSV-1 strain 17 *ts*K.

We are at present investigating by similar transformation experiments to those already described (Cameron *et al.*, 1985) the possible sequence dependence or independence of the upregulation of the cellular polypeptides recognised by the TG7A monoclonal antibody and the relevance of these upregulated polypeptides to the immortalised cell phenotype.

In conclusion, although we have not established that the polypeptides recognised by the TG7A monoclonal antibody represent the initial step in the conversion of primary or secondary cells to an established or immortalised cell line we visualise that HSV upregulates cellular polypeptides which may have a key role in the immortalisation of cells because we can show maintenance of these same upregulated polypeptides in established cell lines.

The infection of cells by HSV-2 causes the accumulation of some cellular polypeptides. Some of these polypeptides appear to define the difference between a primary, secondary or tertiary rat embryo cell and a rat cell which has been immortalised. The function of the genes encoding these proteins may be similar to that of the large T antigen of polyoma virus (Razoulzadegan *et al.*, 1982), the *E*1a region of Adeno 2 (Ruley, 1983) or the c-*myc* oncogene (Land *et al.*, 1983). We are currently attempting to investigate the role that HSV may play in the immortalisation of cells. The concept of oncogenes which cooperate is now well established, e.g., the immortalising role of *E*1a which can cooperate with genes such as Ras (Newbold *et al.*, 1982; Newbold and Overell, 1983) to produce the fully transformed phenotype. This study shows that normal HSV upregulates cellular polypeptides which are also upregulated in transformed and immortalised cells. Other genes, perhaps of viral origin, may cooperate to produce an oncogenically transformed cell.

Materials and methods

Control cells

Rat embryo cells from inbred Hooded Lister rats were prepared as described (Macnab, 1974, 1975, 1979). The rat embryo cells were used as secondary or occasionally as tertiary control cultures.

HSV-transformed Hooded Lister rat embryo cells

Bn3 and Bn5 cells (called B3 and B5 in the Figures) transformed by the cloned *Bgl*II n fragment of HSV-2 strain HG52, Bn5T cells (called B5 in the Figures) were cultured from a tumour induced by injection of Bn5 cells (Cameron *et al.*, 1985). REα and RE2A, two independent cell lines transformed by the sheared DNA of HSV-1 strain HFEMα (Wilkie *et al.*, 1974; Macnab, unpublished results; Park and Macnab, 1983); RE Asyn$^+$C transformed by HSV-1 strain 17 *ts* Asyn$^+$ at NPT (Büültjens and Macnab, 1981), RE1 and RE7 transformed by HSV-2 strain HG52 *ts*1 or *ts*7 at NPT respectively (Macnab, 1974; Macnab *et al.*, 1980; Park *et al.*, 1980). ICTI transformed by HSV-2 strain HG52 at supraoptimal temperature (Cameron *et al.*, 1985).

Immortalised rat embryo cell lines

Hood cell lines are a series of Hooded Lister rat embryo cells which have been established by us in culture (Macnab, 1979; Macnab *et al.*, 1980; Büültjens and Macnab, 1981) to serve as immortalised control cells for HSV transformation experiments. Rat-1 cells are an established contact inhibited Fischer rat cell line F2408 (Prasad *et al.*, 1976).

RSV-transformed cells

Two different lines were used (i) Rat-1 cells (called R1 in Figure 3) transformed by wild-type RSV and (ii) Rat-1 cells transformed by RSV *ts* LA24, a mutant *ts* in src (Steimer and Klagsbrun, 1981) and called LA in these studies.

Adeno 12 transformed cells

Hooded Lister rat cells transformed by Adeno 12 (Ad 12) were used. In one line AD12 DNA sequences were retained and in the other line AD12 DNA sequences were no longer detectable by Southern blotting analysis (Paraskeva *et al.*, 1982). All cells were cultivated in BHK21 medium supplemented by 10% fetal calf serum, 2 mM glutamine, 100 μg/ml streptomycin and 100 units/ml penicillin (Gibco, Paisley, Scotland).

Sera

Sera from tumour-bearing animals (TBS) were collected from rats bearing tumours to Bn5 cells or Bn5T cells. Antisera to HSV-2 infected cell polypeptides were prepared by inoculation of rats with sonicated extracts of rat embryo cells infected for 7.5 h at 10 p.f.u./cell with HSV-2 strain HG52 in Freund's complete adjuvant. The monoclonal antibody which recognises cell-encoded polypeptides of mol. wts. 90 000 and 40 000, was TG7A generated and characterised by

La Thangue (La Thangue and Latchman, in preparation). It was raised against mice inoculated with HSV-2 infected cell polypeptides which bound to a DNA affinity column. The method of preparation was similar to that described for other cellular polypeptides upregulated on HSV infection (La Thangue *et al.*, 1984).

The 4H monoclonal antibody used as a control in Figure 1 was raised against an unrelated polypeptide.

Radiolabelling and immunoprecipitation

Cells for polypeptide analysis or radio immunoprecipitation were labelled with 50 μCi/ml [^{35}S]methionine (Amersham International) in medium containing one fifth of the normal concentration of methionine. For polypeptide profile analysis (Marsden *et al.*, 1978) cells were thoroughly washed and lysed in a mixture of glycerol, 5% SDS and gel buffer containing β-mercaptoethanol. For immunoprecipitation studies cells were lysed in RIPA buffer (Docherty *et al.*, 1981) spun at 14 000 *g* for 15 min and immunoprecipitated by a modification of this method and that of Showalter *et al.* (1981). Briefly, the lysed cells were pre-absorbed with rabbit anit-mouse IgG and *S. aureus* protein A for 1 h at 4°C prior to immune precipitation with the test antibody and in the case of the monoclonal TG7A, with rabbit antimouse antibody also. After spinning, immune complexes were adsorbed to *S. aureus* protein A and after three washes were eluted in buffer at 75°C. Polypeptides and immune complexes were separated on 6.5–15% SDS polyacrylamide gels, treated with En-hance (New England Nuclear) and dried under vacuum before autoradiography.

Peptide mapping

Peptide mapping was carried out as described by Cleveland *et al.* (1977). Briefly, [^{35}S]methionine-labelled polypeptides were identified by autoradiography on gels dried down without En-hance treatment. Excised bands were rehydrated in sample buffer, electrophoresed through 18% polyacrylamide gels together with *S. aureus* V8 protease (Miles) and digested to completion with 10 μg of protease per digest for 1 h prior to entering the separating gel.

Acknowledgements

We wish to thank Professor J.H.Subak-Sharpe, Professor A.Mitchison and Dr Roger Everett for critically reading this manuscript and Dr Howard Marsden for both critical reading of the manuscript and helpful discussion of the results. In addition, we would like to thank Professor R.O.Hynes of MIT, Boston for encouraging discussion and for providing the Rat 1 and RSV-transformed cell lines, and Dr Phil Gallimore of the Department of Cancer Studies, University of Birmingham, UK, for providing the AD12-transformed cell lines and their appropriate controls. Dr N.B.La Thangue was the recipient of a SERC Case Award.

References

Adam,E., Kaufman,R.H., Melnick,J.L., Levy,A.H. and Rawls,W.E. (1973) *Am. J. Epidemiol.*, **98**, 77-83.

Boshart,M., Gissmann,L., Ikenberg,H., Kleinheinz,A., Scheurlen,W. and zur Hausen,H. (1984) *EMBO J.*, **3**, 11151-1157.

Burns,J.C. and Murray,B.K. (1981) *J. Gen. Virol.*, **55**, 305-313.

Büültjens,T.E.J. and Macnab,J.C.M. (1981) *Cancer Res.*, **41**, 2540-2547.

Cameron,I.R. and Macnab,J.C.M. (1980) in Nahmias,A.J., Dowdle,W.R. and Schninazi,R.F. (eds.), *The Human Herpes Viruses*, Elsevier North Holland Inc., NY, pp. 570-572.

Cameron,I.R., Park,M., Dutia,B.M., Orr,A. and Macnab,J.C.M. (1985) *J. Gen. Virol.*, **66**, 517-527.

Cleveland,D.W., Fischer,S.G., Kirschner,M.W. and Laemmli,U.K. (1977) *J. Biol. Chem.*, **252**, 1102-1106.

Docherty,J.J., Subak-Sharpe,J.H. and Preston,C.M. (1981) *J. Virol.*, **40**, 126-132.

Dürst,M., Gissmann,L., Ikenberg,H. and zur Hausen,H. (1983) *Proc. Natl. Acad Sci. USA*, **80**, 3812-3815.

Everett,R.D. and Dunlop,M. (1984) *Nucleic Acids Res.*, **12**, 5969-5978.

Galloway,D.A. and McDougall,J.K. (1981) *J. Virol.*, **38**, 749-760.

Galloway,D.A. and McDougall,J.K. (1983) *Nature*, **302**, 21-24.

Gissmann,L., Wolnik,L., Ikenberg,H., Kildovsky,U., Schnurch,H.G. and zur Hausen,H. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 560-563.

Jariwalla,R.J., Aurelian,L. and Ts'o,P.O.P. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 2279-2283.

Land,H., Parada,L.F. and Weinberg,R.H. (1983) *Nature*, **304**, 596-602.

LaThangue,N.B., Shriver,K., Dawson,C. and Chan,W.L. (1984) *EMBO J.*, **3**, 267-277.

Macnab,J.C.M. (1974) *J. Gen. Virol.*, **24**, 143-153.

Macnab,J.C.M. (1975) in de The,G., Epstein,M.A. and zur Hausen,H. (eds.), *Oncogenesis and Herpes viruses II*, Lyon, France, pp. 227-236.

Macnab,J.C.M. (1979) *J. Gen. Virol.*, **43**, 39-56.

Macnab,J.C.M. and McDougall,J.K. (1980) in Nahmias,A.J., Dowdle,W.R. and Schninazi,R.F. (eds.), *The Human Herpes Viruses*, Elsevier North Holland Inc., p. 634.

Macnab,J.C.M., Visser,L., Jamieson,A.T. and Hay,J. (1980) *Cancer Res.*, **40**, 2074-2097.

Macnab,J.C.M., Park,M., Cameron,I.R., Kitchener,H.C., Walkinshaw,S.A., McLauchlan,J., Davison,M.-J., Cordiner,J.W., Clements,J.B. and Subak-Sharpe,J.H. (1985) in Grundmann,E. (ed.), *Cancer Campaign*, Gustav Fischer Verlag, Stuttgart, New York, pp. 151-160.

McLauchlan,J. and Clements,J.B. (1983) *EMBO J.*, **2**, 1953-1961.

Marsden,H.S., Stow,N.D., Preston,V.G., Timbury,M.C. and Wilkie,N.M. (1978) *J. Virol.*, **28**, 624-642.

Naib,Z.M., Nahmias,A.J. and Josey,W.E. (1966) *Cancer*, **19**, 1026-1031.

Newbold,R.F. and Overell,R.W. (1983) *Nature*, **304**, 648-651.

Newbold,R.F., Overell,R.W. and Connell,J.R. (1982) *Nature*, **299**, 633-635.

Notarianni,E.L. and Preston,C.M. (1982) *Virology*, **123**, 113-122.

Paraskeva,C., Brown,R.W., Dunn,A.R. and Gallimore,P.H. (1982). *J. Virol.*, **44**, 759-764.

Park,M., Lonsdale,D.M., Timbury,M.C., Subak-Sharpe,J.H. and Macnab,J.C.M. (1980) *Nature*, **285**, 412-415.

Park,M., Kitchener,H.C. and Mcnab,J.C.M. (1983) *EMBO J.*, **2**, 1029-1034.

Park,M. and Macnab,J.C.M. (1983) *J. Gen. Virol.*, **64**, 755-758.

Prasad,I., Zouzias,D. and Basilico,C. (1976) *J. Virol.*, **18**, 436-444.

Razoulzadegan,M., Cowie,A., Carr,A., Glaichenhaus,N., Kamen,R. and Cuzin,F. (1982) *Nature*, **300**, 713-718.

Reyes,G.R., La Femina,R., Hayward,S.D. and Hayward,G.S. (1979) *Cold Spring Harbor Symp. Quant. Biol.*, **44**, 629-641.

Ruley,H.E. (1983) *Nature*, **304**, 602-606.

Showalter,S.D., Zweig,M. and Hampar,B. (1981) *Inf. and Imm.*, **34**, 684-682.

Steimer,K. and Klagsbrun,M. (1981) *J. Cell Biol.*, **88**, 294-300.

Vonka,V., Kanka,J., Hirsch,I., Zavadova,H., Krcmar,M., Scuhankiva,A., Rezacova,D., Broucer,J., Press,M., Domorazkova,E., Svoboda,B., Havrankova,A. and Jelinek,J. (1984) *Int. J. Cancer*, **33**, 61-66.

Wentz,W.B., Reagan,J.W., Heggie,A.D., Fu Yao-shi and Anthoney,D.D. (1981) *Cancer*, **48**, 1783-1790.

Wentz,W.B., Heggie,A.D., Anthoney,D.D. and Reagan,J.W. (1983) *Science (Wash.)*, **222**, 1128-1129.

Wilkie,N.M., Clements,J.B., Nacnab,J.C.M. and Subak-Sharpe,J.H. (1974) *Cold Spring Harbor Symp. Quant. Biol.*, **39**, 657-666.

Walker,A.I., Hunt,T., Jackson,R.J. and Anderson,C.W. (1985) *EMBO J.*, **4**, 139-145.

zur Hausen,H. (1982) *Lancet*, **II**, 1370-1372.

Received on 15 July 1985; revised on 9 September 1985