JOURNAL OF VIROLOGY, Jan. 1977, p. 284-291
Vol. 21, No. 1

Printed in U.S.A.

Expression of an Early, Nonstructural Antigen of Herpes Simplex Virus in Cells Transformed In Vitro by Herpes Simplex Virus

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Received for publication 26 July 1976

Hyperimmune rabbit antiserum to an early, nonstructural herpes simplex virus type 2 (HSV-2)-induced polypeptide (VP143) reacted in immunofluorescence tests with a variety of cell lines transformed by HSV-2. Cytoplasmic fluorescence was observed in 10 to 50% of HSV-2-transformed cells, whereas no fluorescence was observed in cells transformed by other oncogenic DNA viruses or by a chemical carcinogen. VP143-specific reactivity could be adsorbed from anti-VP143 serum with HSV-2-transformed cells but not with cells transformed by other agents. When HSV-2-transformed cells were synchronized in mitosis and examined at various times postmitosis for VP143-specific fluorescence, the expression of VP143 was shown to be cell cycle dependent.

Herpes simplex virus (HSV) has been serologically associated with human malignancy (24, 26). The demonstration of in vitro oncogenic transformation of mammalian cells with HSV types 1 (6) and 2 (5, 16) represents significant supportive evidence for the in vivo oncogenic potential of these viruses.

Definitive proof that transformed cells contain viral information lies in the demonstration of viral DNA or its products, viral RNA, and virus-specific proteins. Viral RNA (2) and, more recently, viral DNA (4, 8, 17, 22) have been identified by molecular hybridization techniques in cells transformed in vitro by HSV type 2. Virus-specific proteins have been demonstrated in HSV-transformed cells by a number of techniques. (i) HSV-specific antigens have been detected by immunofluorescence tests using hyperimmune antisera to HSV (1, 5, 6, 16, 18-20, 27, 30). (ii) Antibodies which react with HSV antigens in neutralization tests have been demonstrated in the sera of animals bearing tumors induced by HSV-transformed cells (1, 5, 16). (iii) The presence of HSV-specific thymidine kinase (TK) has been demonstrated in TK^- mouse L cells after in vitro transformation with HSV (23). (iv) Complementation of HSV type 2 temperature-sensitive mutants in HSV type 2-transformed cells at the nonpermissive temperature has been demonstrated (15, 21). Although viral genetic information has been shown to be present in HSV-transformed cells, the identity of the specific viral genes involved in the induction and maintenance of transformation is currently not known. Clearly, studies of viral gene expression in transformed cells would benefit greatly from the use of techniques that are capable of detecting individual viral gene products in these cells. Of the four methods just described for detecting viral proteins, only the latter two are capable of detecting the product of a single viral gene.

Courtney and Benyesh-Melnick (3) have recently demonstrated the usefulness of hyperimmune antiserum prepared against an individual HSV-specific polypeptide (VP175) for studies of the expression of this polypeptide in virus-infected cells. The use of this antiserum in indirect immunofluorescence tests has facilitated studies of the kinetics of synthesis and the intracellular location of an individual viral gene product in virus-infected cells. The potential usefulness of such polypeptide-specific antiserum in studies of HSV-transformed cells was first reported by Kimura et al. (16). In these studies hyperimmune antiserum prepared against a nonstructural HSV-specific polypeptide (VP143) synthesized early in the infectious cycle was used to demonstrate the presence of VP143 in fixed preparations of HSV-transformed cells.

In the present report we have attempted to further characterize the expression of VP143 in HSV-transformed cells with regard to the sensitivity and specificity of the reaction and as a function of the stage of the cell cycle during which maximum expression occurs.

MATERIALS AND METHODS

Cells and cell culture. Primary hamster embryo fibroblasts were obtained from inbred Lakeview

Syrian hamster embryos (strain LSH) in the thirteenth day of gestation (Lakeview Hamster Colony, Newfield, N.J.). Cultures were used in low passage (passages 1 to 5, LSH lp) and high passage (passages 39 to 86, LSH hp). An HSV type 2 (HSV-2)-transformed LSH tumor cell line (line 333-8-9 Tu) was kindly provided by F. Rapp and R. Duff (Milton S. Hershey Medical Center, Hershey, Pa.). The cell line was received in high passage (>100). It was passed one additional time in hamsters and four times in cell culture before use in this study. Additional transformed LSH cell lines and tumor (Tu) cell lines derived from them were kindly supplied by S. Tevethia (Tufts University School of Medicine, Boston, Mass.). These lines include the following: LSH-DMBA Tu (passages 8–15), LSH-SV40 Tu (passages 6-27), LSH-Ad12 (passage 3), and LSH-Ad7 Tu(passage 9) transformed by dimethyl-benzanthracene (DMBA), simian virus type 40 (SV40), adenovirus 12, and adenovirus 7, respectively. A laboratory strain of BHK-21 cells in passage 30 was also used in this study. All cells were grown in Eagle medium supplemented with 10% fetal bovine serum and 0.075% $NaHCO_3$ for cultures in closed vessels and 0.225% $NaHCO_3$ for cultures in open vessels.

Preparation of antiserum. Two types of antisera were used in these studies: hyperimmune rabbit antiserum to HSV-2 was prepared as described by Esparza et al. (7) and antiserum to VP143 was prepared as described by Courtney and Benyesh-Melnick (3). It should be noted that this polypeptide was previously designated as VP134 based on the molecular weight determination of HSV type 1 polypeptides. The corresponding polypeptide in HSV-2-infected cells has a slightly higher molecular weight and will be subsequently designated as VP143. Briefly, the latter antiserum was prepared as follows. The nuclear fraction of HSV-2 (strain 186)-infected human embryonic lung fibroblasts harvested 24 h postinfection was subjected to sodium dodecyl sulfate preparative polyacrylamide gel electrophoresis. Polypeptide VP143 was removed from peak fractions and further purified by two additional runs on analytical cylindrical gels. Rabbits were immunized with gel slices containing VP143 as previously described (3).

Immunofluorescence test. The indirect immunofluorescence (IF) test described by Porter et al. (25) was employed with minor modifications. Approximately 5×10^4 cells were seeded on cover slips. After incubation for 6 h at 37°C, cover slips were washed three times in Tris, pH 7.4, dried for 25 min at room temperature, and fixed in cold acetone (4°C) for 15 min. Before staining, cells were rehydrated with 0.1 ml of Tris for 5 min at room temperature. Cells were then treated for 30 min at room temperature with either antisera or preimmune rabbit serum diluted 1:2 in Tris. Cover slips were then washed three times in Tris and treated for 30 min with fluorescein-conjugated goat anti-rabbit gamma globulin (Hyland, Div. of Travenol Laboratories, Inc., Costa Mesa, Calif.). Cover slips were washed three times in Tris and once in distilled water, air dried, and mounted in Elvanol. Control, infected cultures were prepared by inoculating primary LSH cells on cover slips with HSV-2 (strain 333) at a multiplicity of 5 PFU/cell. After a 4-h incubation at 37°C, cover slips were processed and stained as described above.

Absorption technique. LSH hp, LSH-SV40 Tu, and 333-8-9 Tu cells were seeded in 100-mm petri dishes to contain approximately 10^7 cells per dish. After 6 h, cells in three dishes were harvested by scraping into the medium and pelleting. Pellets containing approximately 3×10^7 cells were washed three times in cold Tris and resuspended in 0.4 ml of undiluted rabbit anti-VP143 serum. Suspensions were then subjected to sonic oscillation in a Raytheon sonic oscillator at 10 kc for two 30-s intervals. Suspensions were shaken gently in a water bath at 37°C for 1 h and centrifuged at $100,000 \times g$ for 20 min. Supernatant fluids were removed, diluted 1:2 in Tris to yield a total of 0.8 ml, and absorbed a second time for 1 h at 37°C and overnight at 4°C with cell pellets containing 6.0×10^7 cells. Suspensions were then spun at $100,000 \times g$ for 20 min, and absorbed sera were tested for VP143 reactivity by IF.

VP143 expression in synchronized 333-8-9 Tu cells. (i) Determination of cell doubling time. Approximately 2×10^5 cells from asynchronous 333-8-9 Tu cultures were used to seed 60-mm petri dishes. At 6-h intervals postseeding, duplicate monolayers were trypsinized, and cells were counted until the number of cells had doubled.

(ii) Preparation of synchronized cell cultures. Suspensions of synchronous cells were prepared by the method of Terashima and Tolmach (31). This method is based upon the observation that cells in mitosis adhere less strongly to the culture substrate and can therefore easily be detached and selectively removed from the monolayer. Therefore, plastic flasks (150 cm^2) were seeded with 10^7 cells, and the cultures were incubated at 37°C for an interval equivalent to one doubling time. At this time mitotic cells were detached from the vessel surface by vigorous shaking and pelleted by low-speed centrifugation. Pellets were held in an ice bath overnight, counted, diluted, and seeded in culture dishes the following morning. Examination of cell suspensions at the time of seeding revealed that approximately 98% of cells were viable and that the nuclei of greater than 90% of cells exhibited mitotic figures.

(iii) Expression of VP143 after synchronization. Approximately 4×10^4 cells synchronized in mitosis were seeded on cover slips. At designated times postseeding, cover slips were processed and examined for IF reactivity using antiserum to VP143.

Determination of S-phase in synchronized 333-8-9 Tu cells. Sixty-millimeter petri dishes were seeded with 5×10^5 333-8-9 Tu cells collected in mitosis. At intervals postseeding, 10 μCi of [^{3}H]thymidine per ml (60 Ci/mM; Schwarz Bio Research, Inc., Orangeburg, N.Y.) was added to each plate. After 30 min, cells were harvested by scraping into the medium, pelleted, washed once with 2 ml of cold TNE (0.01 M Tris-hydrochloride, 0.1 M NaCl, and 0.001 M EDTA, pH 7.4), and repelleted. Cell pellets were resuspended in 3 ml of TNE medium and were lysed by treatment with 0.08 ml of 10% sodium lauryl sarcosinate for 10 min at room temperature. Suspensions were deproteinized by treatment with 0.06 ml of Pronase (1 mg/ml) at 37°C for 30 min. One-tenth mil-

liliter of each suspension was placed on a Whatman GF/A filter disk. Disks were then dried, washed once for 5 min in cold (4°C) 5% trichloroacetic acid, washed twice in cold (4°C) water, redried, and placed in scintillation vials. Ten milliliters of scintillation fluid was added to each vial, and samples were counted in a Beckman LS250 liquid scintillation counter.

RESULTS

Patterns of VP143-specific fluorescence in HSV-infected and HSV-transformed cells. Typical patterns of fluorescence staining observed in HSV-2-infected and -transformed LSH cells treated with antisera to VP143 and to HSV-2 are shown in Fig. 1. When treated with anti-VP143 serum, HSV-2-infected cells exhibited predominantly nuclear fluorescence (Fig. 1A). In contrast, 10 to 50% of HSV-2-transformed cells exhibited distinct cytoplasmic, predominantly perinuclear fluorescence (Fig. 1B). Whereas diffuse cytoplasmic and nuclear fluorescence was observed in HSV-2 infected cells treated with anti-HSV-2 serum (Fig. 1D), no specific fluorescence was observed in HSV-2-transformed 333-8-9 Tu cells treated with this antiserum (Fig. 1E). Thus, while antiserum to HSV-2 failed to produce HSV-specific fluorescence in HSV-2-transformed cells, a distinct pattern of fluorescence was observed in cells treated with antiserum to VP143. Although internal fluorescence was not observed in fixed preparations of HSV-2-transformed cells using anti-HSV-2 serum, virus-specific membrane fluorescence was observed in unfixed preparations of these cells using anti-HSV-2 serum (16).

Specificity of the reaction. (i) Reaction of anti-VP143 serum with LSH cells transformed by other agents. To test the specificity of the VP143 reaction, four HSV-2-transformed LSH cell lines and four LSH cell lines transformed by other agents were tested for reactivity to VP143 antiserum (Table 1). A positive cytoplasmic, predominantly perinuclear reaction was observed in the four HSV-2-transformed cell lines; no reaction was observed in cells transformed by SV40 (Fig. 1C), adenovirus 7, adenovirus 12, or a known chemical carcinogen (dimethylbenzanthracene). No VP143-specific reactivity was observed in either low- or high-passage LSH cells or in a common laboratory line of hamster cells, BHK-21 (not shown).

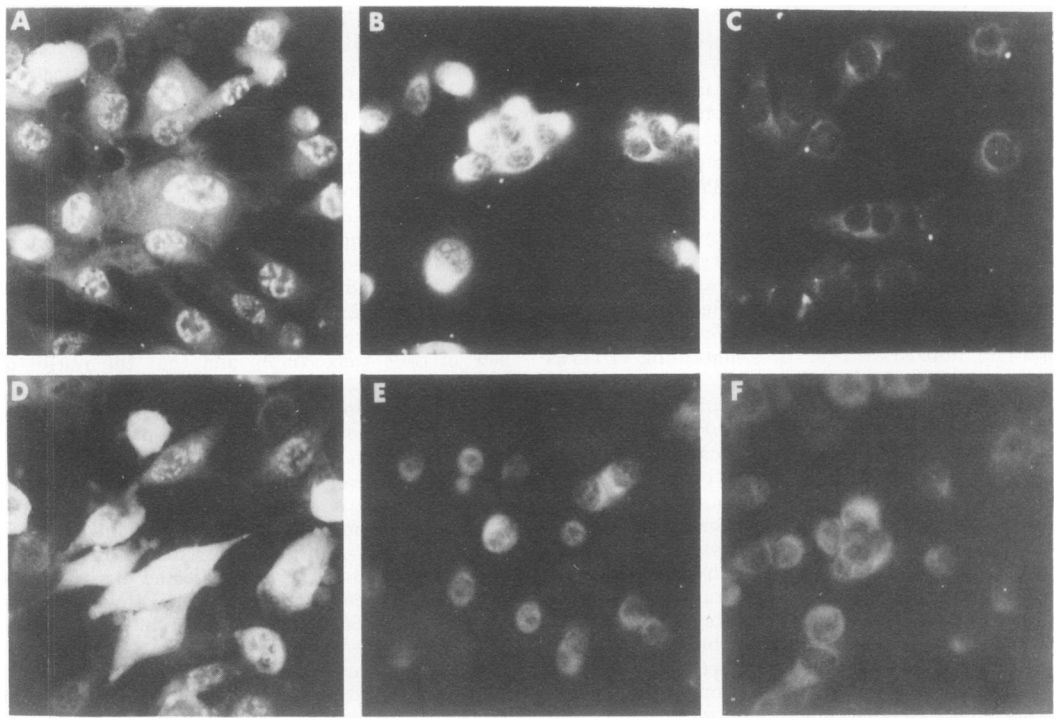

FIG. 1. *Patterns of IF staining observed in HSV-2-infected, virus-transformed and normal hamster cells after treatment with anti-VP143 and anti-HSV-2 sera. (A) HSV-2 infected cells stained with anti-VP143 serum 4 h postinfection. (B) 333-8-9 Tu cells stained with anti-VP143 serum. (C) LSH-SV40 Tu cells stained with anti-VP143 serum. (D) HSV-2-infected cells stained with anti-HSV-2 serum 4 h postinfection. (E) 333-8-9 Tu cells stained with anti-HSV-2 serum. (F) 333-8-9 Tu cells stained with preimmune rabbit serum.*

(ii) Absorption experiments. In an effort to further demonstrate the specificity of the VP143 reaction, antiserum to VP143 was absorbed with 333-8-9 Tu, LSH-SV40 Tu, and LSH hp cells and then tested for HSV-specific reactivity in transformed cells. Typical patterns of fluorescent staining before and after absorption are shown in Fig. 2. The reactivity of anti-VP143 serum with 333-8-9 Tu cells (Fig. 2A) was eliminated after absorption with homologous cells (Fig. 2B); however, only a slight decrease in the intensity of staining was observed after antiserum was adsorbed with LSH SV40 Tu (Fig. 2C) or with LSH hp (Fig. 2D).

Expression of VP143 in synchronized 333-8-9 Tu cells. Preliminary studies had indicated that the expression of the antigen in HSV-transformed 333-8-9 Tu cells which reacted with antisera to VP143 was cell cycle dependent: the intensity of staining in cells was observed to be greater 6 h after trypsinization and seeding than after 12 to 18 h. To examine the expression of VP143 as a function of the stage of the cell cycle, it was first necessary to determine the doubling time of 333-8-9 Tu cells. The number of 333-8-9 Tu cells doubled within 30 h after seeding (Fig. 3). Therefore, 333-8-9 Tu cells were collected in mitosis, seeded on cover slips, and examined at various times postseeding for HSV-specific fluorescence. The results of this study are summarized in Fig. 4. The maximum number of cells which exhibited fluorescence in the 30-h test period appeared to occur 6 h postseeding when 90% of cells were positive. Most cells were observed to be in pairs, indicating recent mitosis. In cells tested at 6 h, fluorescence was localized to the perinuclear region (ring-forms), and large, round, perinuclear accumulations were prominent. By 12 h postseeding the number of cells exhibiting fluorescence was reduced (50 to 75%). Furthermore, the staining reaction was also slightly less intense and somewhat more diffuse in the cytoplasm. The intensity of cytoplasmic fluorescence seen at 18 h postseeding was greatly reduced. The reduced intensity of the reaction made quantitation of IF-positive cells more difficult and less reliable. By 24 h perinuclear accumulations and ring-forms were again prominent in approximately 50% of cells, and staining had become more intense. By 24 to 30 h postmitosis perinuclear accumulations predominated, and many cells had begun to divide. Since the intensity and localization of the reaction varied greatly from cell to cell by 30 h, it appeared as if most cells were no longer in synchrony at this time.

Relationship between the expression of VP143 and DNA synthesis in synchronized 333-8-9 Tu cells. To determine the relationship between the expression of VP143 and the time of S-phase in the cell cycle, 333-8-9 Tu cells were collected in mitosis, seeded in culture dishes, pulsed with [^{3}H]thymidine, and harvested in parallel with cover slips prepared for immunofluorescence staining. Maximum incorporation of [^{3}H]thymidine into DNA occurred approximately 18 h postmitosis, whereas maximum intensity of the VP143 fluorescence reaction occurred from 6 to 12 h and from 20 to 30 h (Fig. 4). The results of this study therefore indicate that the intensity of the VP143 staining reaction is cell cycle dependent and that maximum intensity occurs before and after the time of maximum DNA synthesis (i.e., during G and early S phase as well as during late S and G_2) of the 30-h 333-8-9 Tu cell cycle.

TABLE 1. *Reaction of anti-VP143 serum with LSH cells transformed by HSV-2 and other agents*

Cell type	Reaction with VP143 antiserum
HSV-2-infected LSH[a]	+ (Nuclear)
333-8-9 Tu (333)[b]	+ (Cytoplasmic and perinuclear)
U-15 Tu (186)	+ (Cytoplasmic and perinuclear)
U4V-8 Tu (333)	+ (Cytoplasmic and perinuclear)
B20V-9 Tu (333)	+ (Cytoplasmic and perinuclear)
LSH-SV40 Tu	–
LSH-Ad7 Tu	–
LSH-Ad12 Tu	–
LSH-DMBA	–
LSH lp	–
LSH hp	–
BHK-21	–

[a] LSH lp cells were infected with HSV-2 strain 333 and harvested 4 h postinfection.

[b] Numbers in parentheses indicate the strain of HSV-2 used to induce transformation. Lines U-15 Tu, U4V-8 Tu, and B20V-9 Tu have been described previously (16).

DISCUSSION

The present study demonstrates the potential usefulness of antiserum prepared against an individual viral polypeptide for studies of viral gene expression in HSV-transformed cells. The specificity of the reaction between anti-VP143 serum and the antigen with which it reacts in HSV-transformed cells has been demonstrated, since HSV-transformed hamster cells gave positive reactions with VP143 antiserum whereas cells transformed by other agents did not. Furthermore, anti-VP143 serum reactivity in HSV-2-transformed cells was significantly decreased after absorption with homologous cells but not with normal cells or with cells transformed by SV40, indicating that the antiserum reacted with a gene product(s) expressed specifically in

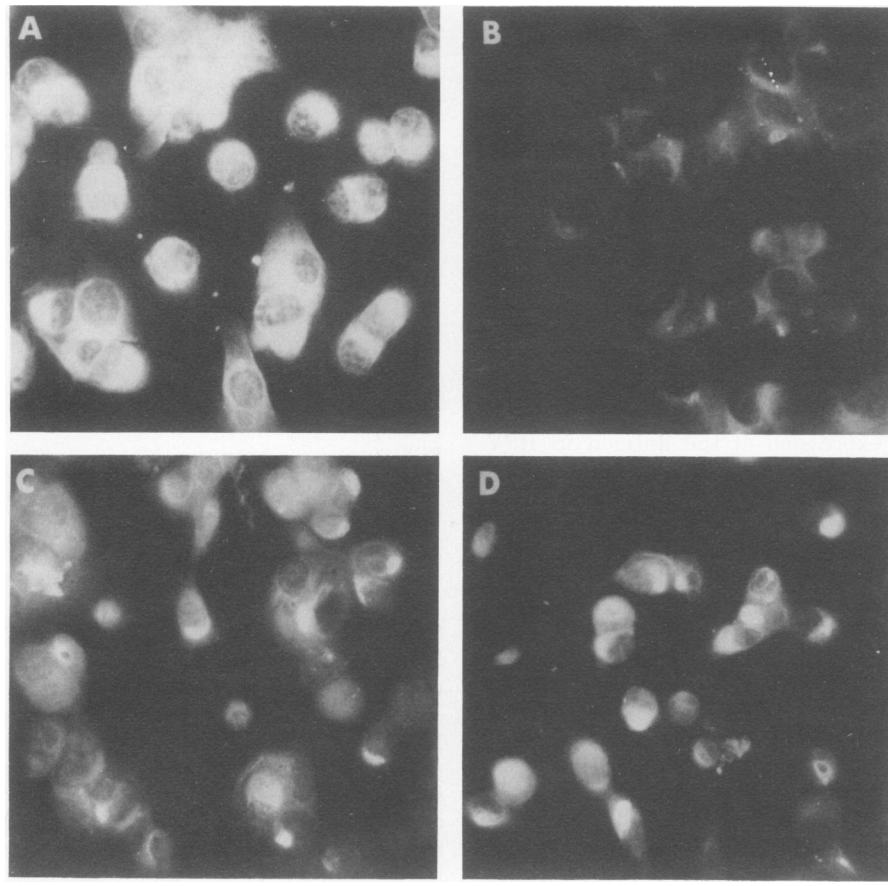

FIG. 2. *Effects of absorption on VP143-specific reactivity in 333-8-9 Tu cells. IF staining observed in 333-8-9 Tu cells treated with anti-VP143 serum that was: (A) unabsorbed, (B) absorbed with 333-8-9 Tu cells, (C) absorbed with LSH-SV40 Tu cells, and (D) absorbed with LSH lp cells.*

HSV-transformed cells. In addition, preliminary results of VP143 IF reactivity in HSV-2-transformed human embryonic lung cells lend further support to the HSV-specific nature of the reaction.

Whether the antigen in HSV-transformed cells that reacts with anti-VP143 serum is identical to the antigen synthesized in HSV-infected cells is not known. Alternative possibilities for the origin of VP143-reactive antigen in HSV-2 transformed cells include the following: (i) the antigen is a virus-modified host protein which shares common antigenic determinants with the infected cell protein; (ii) the antigen is a virus-coded precursor protein which shares antigenic determinants with the viral protein produced in infected cells; or (iii) the antigen is a cellular gene product which is absent in normal cells and derepressed after infection and transformation by HSV. Of these three possibilities, (iii) is the least likely, since derepression of this cellular gene must occur only after infection and transformation by HSV. The definitive answer regarding the origin and nature of VP143 reactive antigen can be found only when the antigens in infected and transformed cells

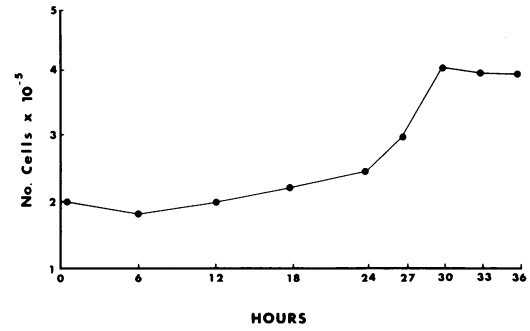

FIG. 3. *Growth kinetics of 333-8-9 Tu cells. Approximately 2 × 10⁵ 333-8-9 Tu cells were seeded into 60-mm petri dishes. Cultures were trypsinized, and cells were counted at the designated times.*

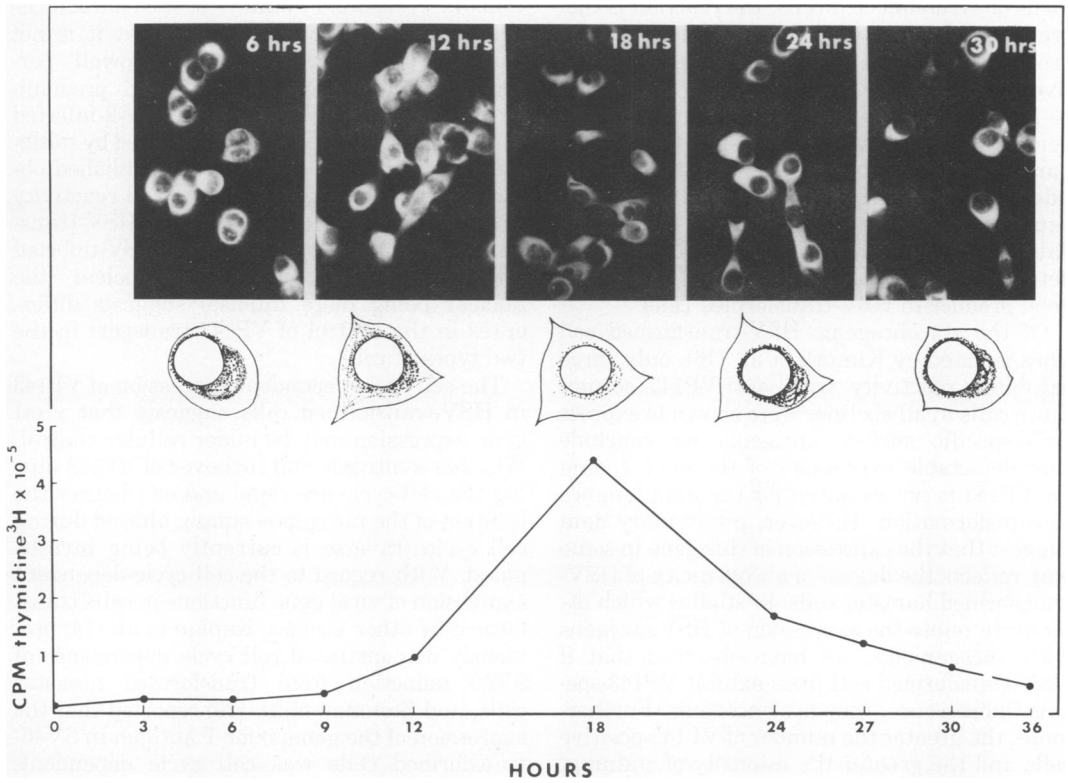

FIG. 4. *VP143 expression and cellular DNA synthesis in synchronized 333-8-9 Tu cells. Cells were synchronized in mitosis and examined for their IF reactivity with anti-VP143 rabbit serum from 6 to 30 h postseeding (photographs). Diagrammatic representations of the cytoplasmic location of VP143 reactivity in typical cells at 6-h intervals are shown beneath photographs. The incorporation of [^{3}H]thymidine into cellular DNA in synchronized cells as a function of hours postseeding is shown at the bottom of the figure.*

can be isolated, characterized, and directly compared.

In the present study antiserum to HSV-2 was capable of detecting viral antigens in HSV-infected cells but not in HSV-transformed cells, whereas antiserum to VP143 detected viral antigens in both kinds of cell. The superiority of polypeptide-specific antisera compared with hyperimmune antiviral serum may reflect the following: (i) animals may mount a stronger response when immunized with a single polypeptide than with a variety of polypeptides present in whole virus preparations, and (ii) the sodium dodecyl sulfate-treated polypeptide in the inoculum may unfold in such a way that more unique antigenic sites are exposed, thus amplifying the antigenic response. In this regard, it should be noted that the IF reaction, which is detected within the transformed cell, may be a reaction to the unfolded polypeptide and not to antigenic sites on the native protein. Furthermore, we recognize that the interpretation of IF data is a rather subjective matter and that a more definitive method for quantitation of VP143 reactivity is desirable. In fact, confirmation of IF results by radioimmunoassay is currently in progress.

Other characteristics of anti-VP143 serum include the following. (i) Anti-VP143 serum does not react with the membrane of HSV-2-transformed cells nor does it neutralize HSV-2 infectivity. (To date, only antiserum prepared against HSV-2 VP119, the major envelope glycoprotein, has been found to neutralize viral infectivity.) (ii) Anti-VP143 serum does react with an antigen(s) in HSV-1-transformed cells, although the IF reactivity is slightly less intense. When anti-VP143 is reacted with both HSV-1- and HSV-2-infected cells, however, the intensity of nuclear staining is the same. It should be further noted that by radioimmune precipitation this antigen (i.e., VP143) appears to be type common (T. Anzai, personal communication).

Although the use of polypeptide-specific antiserum appears to be a more sensitive means of detecting the presence of viral antigens in transformed cells than is antiserum to whole

virus or virus-infected cells, the reaction is theoretically capable of detecting a single gene product. Consequently, the absence of a positive reaction in HSV-transformed cells cannot be interpreted to indicated the absence of viral gene expression in these cells. Antisera prepared against a series of HSV-specific polypeptides would be essential to screen for HSV-gene expression in transformed cells. Of greater value will be the use of anti-VP143 serum in determining the specific function of the VP143 gene product in HSV-transformed cells.

Of the six oncogenic HSV-transformed cell lines isolated by Kimura et al. (16), only three exhibited reactivity with anti-VP143 serum. Since cells in all six lines were shown to express HSV-specific surface antigens, we conclude that detectable expression of the gene coding for VP143 is not essential for the maintenance of transformation. However, preliminary data suggest that the expression of this gene in some way reflects the degree of oncogenicity of HSV-transformed hamster cells: in studies which attempt to relate the expression of HSV antigens with oncogenicity, we have observed that if HSV-transformed cell lines exhibit VP143-specific fluorescence, they are oncogenic. Furthermore, the greater the number of VP143-positive cells and the greater the intensity of staining, the more oncogenic the cell lines have proven to be (unpublished observation). HSV-transformed cell lines that do not express VP143 have been observed to be non-oncogenic or only weakly oncogenic.

Using hyperimmune hamster antisera to HSV in IF tests, Duff and Rapp reported that a small proportion of the cells in several HSV-transformed cell lines contained HSV-specific antigens (5, 6). Other investigators have reported both internal and surface fluorescence in HSV-transformed cells using hyperimmune anti-HSV serum (18–20, 27). However, in our hands the reproducibility and sensitivity of IF tests for detecting internal antigens using hyperimmune hamster and rabbit antisera to HSV have been poor (16; unpublished observations).

Studies of the expression of an individual viral gene in HSV-transformed cells have to date been limited to studies of viral thymidine kinase synthesis. The present report is therefore the first to describe studies of a virus-specific gene product in HSV-transformed cells employing IF techniques. The HSV-specific polypeptide, VP143, is synthesized during the early stages (2 to 6 h postinfection) of virus replication. Based on the Honess and Roizman (13) classification of HSV-1 polypeptides, HSV-2 VP143 would be classified as a beta polypeptide (3). Preliminary studies of the characteristics of this polypeptide suggest that it is not found in purified virions (K. L. Powell, personal communication), it is found predominantly in the nuclear fraction of HSV-2-infected cells, and its production is not affected by inhibitors of viral DNA synthesis (unpublished observations). The finding that VP143 reactivity was restricted to the cytoplasm of HSV-transformed cells but that reactivity in HSV-infected cells was both cytoplasmic and nuclear (the nuclear being more intense) suggests differences in the control of VP143 transport in the two types of cell.

The cell cycle-dependent expression of VP143 in HSV-transformed cells suggests that viral gene expression may be under cellular control. Whether synthesis and turnover of VP143 during the cell cycle are rapid and/or whether the location of the antigen is simply altered during cell cycle traverse is currently being investigated. With regard to the cell cycle-dependent expression of viral gene functions in cells transformed by other viruses, Kaplan et al. (14) previously demonstrated cell cycle dependence of SV40 induction from transformed hamster cells, and Stenman et al. (29) reported that the expression of the gene(s) for T antigen in SV40-transformed cells was cell cycle dependent. Similarly, Greenberger and Aaronson (9) and Schwartz et al. (28) presented evidence to suggest the cell cycle dependence of drug-induced activation of type-C RNA viruses. Precedent for the cell cycle-dependent expression of antigens in cells transformed by herpesviruses comes from studies by Hampar et al., who described the cell cycle-dependent activation of Epstein-Barr virus antigens (10–12).

Further studies are currently in progress to elucidate the function of VP143 in both HSV-infected and -transformed cells and to examine human cervical carcinoma cells for the presence of VP143-specific reactivity. Preliminary results of studies with cervical carcinoma cells have demonstrated that 2 of 13 lines so far examined express VP143 in a pattern similar to that described for hamster cells transformed in vitro by HSV-2 (unpublished data with R. Lewis, E. Adam, and J. L. Melnick). The use of polypeptide-specific antiserum in studies of the synthesis of other viral gene products in transformed cells is also in progress.

ACKNOWLEDGMENT

This investigation was supported by research contract no. NO1 CP 53526 within the Virus Cancer Program of the National Cancer Institute.

LITERATURE CITED

1. Boyd, A. L., and T. W. Orme. 1975. Transformation of

mouse cells after infection with ultraviolet irradiation-inactivated herpes simplex virus type 2. Int. J. Cancer 16:526-538.
2. Collard, W., H. Thorton, and M. Green. 1973. Cells transformed by human herpesvirus type 2 transcribe virus-specific RNA sequences shared by herpesvirus types 1 and 2. Nature (London) New Biol. 243:264-266.
3. Courtney, R. J., and M. Benyesh-Melnick. 1974. Isolation and characterization of a large molecular-weight polypeptide of herpes simplex virus type 1. Virology 62:539-551.
4. Davis, D. B., and D. T. Kingsbury. 1976. Quantitation of the viral DNA present in cells transformed by UV-irradiated herpes simplex virus. J. Virol. 17:788-793.
5. Duff, R., and F. Rapp. 1971. Properties of hamster embryo fibroblasts transformed in vitro after exposure to ultraviolet-irradiated herpes simplex virus type 2. J. Virol. 8:469-477.
6. Duff, R., and F. Rapp. 1973. Oncogenic transformation of hamster embryo cells after exposure to inactivated herpes simplex virus type 1. J. Virol. 12:209-217.
7. Esparza, J., D. J. M. Purifoy, P. A. Schaffer, and M. Benyesh-Melnick. 1974. Isolation, complementation and preliminary phenotypic characterization of temperature-sensitive mutants of herpes simplex virus type 2. Virology 57:554-565.
8. Frenkel, N., H. Locker, B. Cox, B. Roizman, and F. Rapp. 1976. Herpes simplex virus DNA in transformed cells: sequence complexity in five hamster cell lines and one derived hamster tumor. J. Virol. 18:885-893.
9. Greenberger, J. S., and S. A. Aaronson. 1975. Cycloheximide induction of xenotropic type C virus from synchronized mouse cells: metabolic requirements for virus activation. J. Virol. 15:64-70.
10. Hampar, B., J. G. Derge, L. M. Martos, M. A. Tagamets, S.-Y. Chang, and M. Chakrabarty. 1973. Identification of a critical period during the S phase for activation of the Epstein-Barr virus by 5-iododeoxyuridine. Nature (London) New Biol. 244:214-217.
11. Hampar, B., J. G. Derge, and S. D. Showalter. 1974. Enhanced activation of the repressed Epstein-Barr viral genome by inhibitors of DNA synthesis. Virology 58:298-301.
12. Hampar, B., G. Lenoir, M. Nonoyama, J. G. Derge, and S.-Y. Chang. 1976. Cell cycle dependence for activation of Epstein-Barr virus by inhibitors of protein synthesis or medium deficient in arginine. Virology 69:660-668.
13. Honess, R. W., and B. Roizman. 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. 14:8-19.
14. Kaplan, J. C., L. F. Kleinman, and P. H. Black. 1975. Cell cycle dependence of simian virus 40 induction from transformed hamster cells by ultraviolet irradiation. Virology 68:215-220.
15. Kimura, S., J. Esparza, M. Benyesh-Melnick, and P. A. Schaffer. 1974. Enhanced replication of temperature-sensitive mutants of herpes simplex virus type 2 (HSV-2) at the nonpermissive temperature in cells transformed by HSV-2. Intervirology 3:162-169.
16. Kimura, S., V. L. Flannery, B. Levy, and P. A. Schaffer. 1975. Oncogenic transformation of primary hamster cells by herpes simplex virus type 2 (HSV-2) and an HSV-2 temperature-sensitive mutant. Int. J. Cancer 15:786-798.
17. Kraiselburd, E., L. P. Gage, and A. Weissbach. 1975. Presence of a herpes simplex virus DNA fragment in an L cell clone obtained after infection with irradiated herpes simplex virus. J. Mol. Biol. 97:533-542.
18. Kucera, L. S., and J. P. Gusdon. 1976. Transformation of human embryonic fibroblasts by photodynamically inactivated herpes simplex virus type 2 at supra-optimal temperature. J. Gen. Virol. 30:257-261.
19. Kutinová, L., V. Vonka, and J. Broucek. 1973. Increased oncogenicity and synthesis of herpesvirus antigens in hamster cells exposed to herpes simplex type 2 virus. J. Natl. Cancer Inst. 50:759-766.
20. Macnab, J. C. M. 1974. Transformation of rat embryo cells by temperature sensitive mutants of herpes simplex virus. J. Gen. Virol. 24:143-153.
21. Macnab, J. C. M., and M. C. Timbury. 1976. Complementation of ts mutants of a herpes simplex virus ts-transformed cell line. Nature (London) 262:233-235.
22. Minson, A. C., M. E. Thouless, R. P. Eglin, and G. Darby. 1976. The detection of virus DNA sequences in a herpes type 2 transformed hamster cell line (333-8-9). Int. J. Cancer 17:493-500.
23. Munyon, W., E. Kraiselburd, D. Davis, and J. Mann. 1971. Transfer of thymidine kinase to thymidine kinaseless L cells by infection with ultraviolet-irradiated herpes simplex virus. J. Virol. 7:813-820.
24. Nahmias, A. J., W. E. Josey, Z. M. Naib, C. F. Luce, and B. A. Guest. 1970. Antibodies to herpes virus hominis type 1 and 2 in humans. II. Women with cervical cancer. Am. J. Epidemiol. 91:547-552.
25. Porter, D. D., I. Wimberly, and M. Benyesh-Melnick. 1969. Prevalence of antibodies to EB virus and other herpes-viruses. J. Am. Med. Assoc. 208:1675-1679.
26. Rawls, W. E., W. A. F. Tompkins, and J. L. Melnick. 1969. The association of herpes virus type 2 and carcinoma of the uterine cervix. Am. J. Epidemiol. 89:547-554.
27. Reed, C. L., G. H. Cohen, and F. Rapp. 1975. Detection of a virus-specific antigen on the surface of herpes simplex virus-transformed cells. J. Virol. 15:668-670.
28. Schwartz, S. A., S. Panem, and W. H. Kirsten. 1975. Distribution and virogenic effects of 5-bromodeoxyuridine in synchronized rat embryo cells. Proc. Natl. Acad. Sci. U.S.A. 72:1829-1833.
29. Stenman, S., J. Zeuthan, and N. R. Ringertz. 1975. Expression of SV40 T antigen during the cell cycle of SV40-transformed cells. Int. J. Cancer 15:547-553.
30. Takahashi, M., and K. Yamanishi. 1974. Transformation of hamster embryo and human embryo cells by temperature sensitive mutants of herpes simplex virus type 2. Virology 61:306-311.
31. Terashima, T., and L. J. Tolmach. 1963. Growth and nucleic acid synthesis in synchronously dividing populations of HeLa cells. Exp. Cell Res. 30:344-362.