Analysis of Chromosomes, Nucleic Acids, and Polypeptides in Hamster Cells Transformed by Herpes Simplex Virus Type 2

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ABSTRACT

Syrian hamster embryo fibroblasts were oncogenically transformed by UV-inactivated Herpes simplex virus type 2. Eighteen clones were isolated shortly after transformation occurred. Two clones and their tumor derivatives were studied using several techniques. The karyotype analysis revealed different chromosome patterns in the two clones and a tendency toward hypodiploidy in the tumor derivatives. All of these cell lines were shown by molecular hybridization to contain 40% of the HSV-genome in several copies. The viral DNA sequence complexity was retained in the tumor derivatives, but a decrease in the copy number was observed. Viral RNA’s were detected by in situ hybridization in all the lines that were tested. Viral antigens could be observed in these transformed cells by immunofluorescence. Finally, polypeptide analysis showed three differences between normal and transformed cells.

INTRODUCTION

Since the first reports of oncogenic transformation of hamster cells by HSV types 1 and 2 (10—12), several authors have published similar results using different mammalian cell species and either UV-inactivated virus, viral DNA, or viral temperature-sensitive mutants at a nonpermissive temperature (7, 8, 21—24, 32, 42, 45). All of these publications provided evidence of the oncogenic potential of the HSV which is thought to be associated with the human cervical carcinoma on the basis of seroepidemiological studies (1, 33, 37, 39). The presence of viral products in HSV-transformed cells have been detected by several techniques. For viral antigens, methodologies have included: (a) indirect immunofluorescence using either total anti-HSV immune serum (7, 21—24, 42) or a specific immune serum against one viral polypeptide (14, 38); (b) neutralization of virus or viral antigens by serum of animal-bearing tumors induced by HSV-transformed cells (3, 12, 21); and (c) the complementation test of HSV ts mutants in a HSV-ts mutant transformed cell line at a nonpermissive temperature (26). Although all of these techniques demonstrated the presence of viral antigens in transformed cells, analysis of polypeptides on polyacrylamide gels revealed neither specific viral polypeptides nor any difference in the polypeptide profile between HSV-transformed and control cells (19).

Frenkel et al. (15) and Minson et al. (31) detected by molecular hybridization 8 to 40% of the viral genome in the cells transformed by Rapp and Duff (36). Coppé and McDougall (6), using in situ hybridization, found nucleic acids in most, but not all, clones selected from the 333-8-9 cell line. However, all of their tumor derivatives failed to show any detectable DNA or RNA with this test. The same negative results prevailed when these clones were tested for HSV-DNA by molecular hybridization, although the parental cell line was shown to carry about 40% of the viral genome (31). Thus, such variable features of oncogenically HSV-transformed cells may be indicative of heterogeneity in the transformed cell population and, therefore, raise questions concerning the minimum viral genetic information required for transformation and its exact role and expression in the induction and maintenance of the malignant state.

For a better understanding of the HSV-related oncogenesis, it is important to investigate the viral products and follow their fate in the transformed cells, using in parallel all the techniques described above. We performed transformation experiments on hamster cells by UV-inactivated HSV-2, and we report here data obtained from comparative studies of 2 clones and their tumor derivatives. These cell lines shared common features such as viral DNA content, presence of viral RNA’s, and antigens, in spite of some differences in the level of their expression and the chromosome patterns. Furthermore, polypeptide analysis by SDS-polyacrylamide gel electrophoresis demonstrated for the first time reproducible differences between the transformed and control cells.

MATERIALS AND METHODS

Cell Cultures. The primary cultures of Syrian hamster fibroblasts obtained from 10- to 13-day-old embryos were grown and maintained in MEM supplemented with 10% fetal calf serum, penicillin, (100 units/ml) and streptomycin, (100 μg/ml). The HEP-2 cells, obtained from American Type Culture Collection, were used to grow virus stocks. They were maintained in MEM a medium with 10% fetal calf serum. For virus growth, the serum content was reduced to 2%.

Virus Inactivation. A high-titer stock (2 × 10^6 PFU/ml) of HSV-2 (strain HG-52, kindly provided by the Institute of Virology of Glasgow) was diluted 1:10 in phosphate-buffered saline; 2 ml of the suspension were placed in a 60-mm Petri dish and irradiated by 2 UV Sylvania G15T8 bulbs at a distance of 30 cm with constant agitation. Using these conditions, a 60-min irradiation inactivated 99.9% of the virus.

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4 The abbreviations used are: HSV, Herpes simplex virus; HSV-2, Herpes simplex virus type 2; SDS, sodium dodecyl sulfate; MEM, minimal Eagle’s medium; HEP-2, human epidermoid carcinoma; PFU, plaque-forming units; R-banding, reversal banding; SSC, standard saline citrate (0.15 M sodium chloride; 0.015 M sodium citrate, pH 7.4); HEP, hamster embryo fibroblast.

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Transformation Assay. The hamster embryo fibroblasts (passage 5) were seeded at $1 \times 10^6$ cells/60-mm Petri dish and incubated for 24 hr at 37°C in 5% CO₂ atmosphere. The medium was then removed, and the cells were infected with 0.5 ml of virus solution at a multiplicity of 10 PFU/cell (titer before inactivation). After a 1-hr absorption, the cells were rinsed, and new medium was added. The medium was changed 24 and 48 hrs after infection. The cells were then regularly passaged at 5 to $6 \times 10^5$ cells per Petri dish. As controls, primary embryo fibroblasts (passage 5) were mock infected and treated in the same conditions.

Transformation Tests. To demonstrate the transformed properties of the cells, 2 tests were used. Growth in soft agar was performed as described previously (27). Two and one-half $10^5$ cells/60-mm Petri dish were seeded in soft agar, and the cultures were observed after 2 weeks for colony formation. The tumorigenicity in vivo was tested by s.c. injection of $1 \times 10^5$ cells in 0.1 ml of MEM without serum to newborn hamsters, 48 hr after birth.

Karyotype. The cells for karyotype analysis were prepared following the standard method. Cells were incubated 36 hr after seeding with Colcemid (0.2 μg/ml) for 2 hr. The culture medium was then replaced by 0.8% sodium citrate solution for 20 min. The cells were then detached by shaking the flasks, centrifuged for 5 min at 800 x $g$, and fixed twice for 30 min in methanol:acetic acid (3 volume:1 volume). After centrifugation, the pellet was resuspended in an aliquot of fixative and spread on cold, wet slides. For chromosome identification, we used the R-banding technique (13).

Purification and Labeling of Viral DNA. The HSV-2 virus was grown for 36 to 48 hr on Hep-2 cells, which were infected at a multiplicity of 0.01 PFU/cell. The cells were then harvested, and the pellet was resuspended in 0.01 M Tris, pH 7.4: 1.5 mM MgCl₂:0.01 M KCl:0.5% Nonidet P-40. After Dounce homogenization, the nuclei were discarded and the virus concentrated on 25 ml of 12% sucrose solution in 10 mM Tris (pH 7.4) in a SW27 rotor at 25,000 rpm for 2 hr. The pellet was resuspended in virus buffer (5 ml) Tris, (pH 7.4):10 mM MgCl₂ and treated with 100 μg RNase A per ml and 60 μg DNase I per ml for 60 min at 37°C. The suspension was made 50 mM EDTA, layered on 35 ml of 20 to 60% sucrose gradient, and centrifuged in a SW27 rotor at 25,000 rpm for 1 hr at 5°C. The viral band was diluted in virus buffer and pelleted by centrifugation and then treated successively by 0.5% SDS in virus buffer for 15 min at 60°C and 0.5 mg pronase per ml at 37°C overnight. The DNA was deproteinized by 3 extractions in phenol:chloroform (1 volume:1 volume) and then precipitated with 2 volumes of ethanol. The precipitate was dissolved in DNA buffer [50 mM Tris, (pH 7.4):20 mM EDTA] and centrifuged to equilibrium in a CsCl gradient for 72 hr at 35,000 rpm in a 75 Ti rotor. The viral DNA band was collected, dialyzed against 0.1 x SSC (pH 7.4) and labeled by nick translation (28, 29). Viral DNA, 0.5 to 1 μg, was diluted in 100 μl of 0.05 M Tris, (pH 7.8):5 mM MgCl₂:10 mM dithiothreitol:50 μg of bovine serum albumin per ml:2 nmoles of deoxyribonucleoside triphosphates, labeled either by $^3H$ or $^{32}P$. The reaction was started by adding $10^7$ of g DNase I per ml (Worthington Biochemicals) and 2 units of Escherichia coli DNA polymerase I (Boehringer Manheim Biochemicals). After 1 hr at 15°C, the mixture was twice deproteinized with freshly distilled phenol, and the unincorporated nucleotides were removed by gel filtration on Sephadex G-50 (Pharmacia Fine Chemicals). For in situ hybridization, the 4 deoxynucleoside triphosphates were $^3H$ labeled (New England Nuclear; 30 to 100 Ci/mmol), giving a specific activity of $2.4 \times 10^7$ cpm/μg. For molecular hybridization, 2 of the 4 deoxynucleoside triphosphates (dGTP and dCTP) were $^{32}P$ labeled (New England Nuclear; 300 Ci/mmol); the specific activity was 2 to $5 \times 10^8$ cpm/μg.

In situ Hybridization. The experimental conditions were those described by Copple and McDougall (6) without any denaturation treatment of the cytological preparations. Briefly, the $[3H]$DNA was denatured in 0.1 x SSC at 100°C for 20 min. The solution was adjusted to 2 x SSC (pH 7.4) and $4 \times 10^6$ cpm/μl. Each cytological preparation received 50 μl (2 x $10^6$ cpm) of this solution before being sealed with a coverslip. After incubation at 65°C for 12 hr, the slides were extensively washed and treated by the Si enzyme (20 units/slide) for 1 hr at room temperature. This treatment was followed by extensive washing in Si buffer (0.2 M sodium acetate:0.3 M sodium chloride:3.10^-5 M zinc sulfate, (pH 4.5)), and the slides were dehydrated in ethanol and dried in a vacuum. For autoradiography, the slides were dipped in Kodak NTB2 emulsion (diluted 1:1 with distilled water). After exposition for 3 weeks at 4°C, they were developed in Kodak D19 for 2 min, fixed for 7 min in 30% sodium thiosulfate, and stained with 3% Giemsa for 30 min.

Molecular Hybridization. Reassociation kinetics were followed as described previously (15). Nick translated $^{[32]P}$DNA, (0.5 to 1 ng) and 5 to 6 mg of sheared cell DNA were added per ml of reaction mixture containing 1 μM NaCl:0.1 M sodium phosphate buffer (pH 7:0.02 M EDTA:20% formamide (v/v). The reaction mixture (50 μl) was overlaid with 100 μl of hot paraffin oil, denatured at 117°C for 10 min, and then quickly brought to the incubation temperature. Samples (5 μl) were taken at various time intervals, diluted in 500 μl of ice-cold water, and frozen at -80°C. The single- and double-stranded DNA were separated by hydroxyapatite chromatography (9, 40) using 0.08 and 0.4 μM phosphate buffer. The experimental conditions are fully detailed by Bibor-Hardy et al. (2).

Immunofluorescence Test. The cells were tested by indirect immunofluorescence (14). Normal and transformed cells were grown on coverslips; the infected controls were obtained by infecting cells for 12 hr at a multiplicity of 0.1 PFU/cell. The HSV-2 antiserum (a gift from Dr. F. Colbène-Ganaptn of Pasteur Institute, Paris) was diluted 1:4 in phosphate-buffered saline. The fluorescein-conjugated goat anti-rabbit γ-globulin was purchased from Hyland (Division of Travenol Laboratories) and used at 1:64 dilution.

Protein Analysis. The methods, including culture, infection, radiolabeling, and gradient gel electrophoresis, were fully reported (30). All experiments were performed at 37°C. For labeling of cellular proteins, monolayers of approximately 1 x $10^6$ cells were washed twice and incubated for 5 hr with MEM, containing one fifth the concentration of methionine and 2% calf serum. The cells were then labeled (New England Nuclear; 300 Ci/mmol) for 20 hr. After harvesting, they were analyzed by SDS:polyacrylamide gel electrophoresis. For HSV-infected controls, HEF monolayers were infected with 20 PFU/cell of HSV-2 and labeled for 5 to 24 hr postabsorption with $^{[35]S}$methionine.
RESULTS

Isolation of the Transformed Cells. Since no foci of morphological transformation were observed in the HSV-treated cells, cultures were passaged once a week. After the 16th passage, the virus-treated cultures showed more rapid growth than did the control HEF. Thus, the transformed cells were probably selected without crisis through passage in vitro. To ascertain the transformed character, culture in soft agar was repeatedly tried with the treated cells at different passages. The control HEF were tested at the 16th passage before becoming senescent between the 18th and 20th passages. The test was always negative for the control and positive for the virus-treated cells only at passage 28. The efficiency of colony formation was 9%. Many colonies were isolated and subcultured separately, of which 18 gave rise to clones. We report here the comparative study of 2 clones designated as 620-2 and 620-7 which have cloning efficiencies in soft agar of 66.5 and 28%, respectively.

Tumorigenicity of the Transformed Cells. The malignant properties of the clones were tested by s.c. injection of 1 x 10⁶ cells to whole litters of newborn hamsters 48 hr after birth. All the inoculated animals (9 for 620-2 and 7 for 620-7) developed definite tumors 10 to 12 days after injection. The tumors were encapsulated, and their histological type was that of undifferentiated fibrosarcomas beginning to invade the underlying muscle. Tumor cell lines (620-2T and 620-7T) with the same fibroblastic morphology as the parental cells were established. As a control, using the same conditions, we inoculated 9 newborn hamsters with normal HEF at the 14th passage; none showed evidence of tumors 7 months after injection.

Karyotype Analysis. The 2 clones and their tumor-derived cell lines were analyzed at the following passages: 620-2, passage 32; 620-7, passage 32; 620-2T, passage 5; and 620-7T, passage 9. For each cell type, 35 metaphases were randomly photographed and karyotyped. By the R-banding technique, it was possible to identify all the different pairs (Fig. 1) and certain abnormalities. As shown in Table 1, we found different chromosome patterns for the 2 clones. The 620-2 cells, with a hyperdiploid modal number, showed 2 subclones. One was characterized by a supernumerary acrocentric chromosome (D18) found in 34% of cells (Fig. 1). The other subclone showed a supernumeration in C-group: C13 chromosome was present in 10% of cells, either associated or not to the extra D18 chromosome; an additional C15 chromosome was found in 12% of cells, one third of which was also carrying the extra D18. Seventeen % of the metaphases were polyploid, ranging between 48 and 129 chromosomes. The pseudo- and hypodiploidy were moderate. As previously reported (6, 34), we found marker chromosomes in 20% of cells; deleted chromosomes were identified in 4 cells (Xp-, A3p-, A4p-, and A4 ring), whereas in the other 3 cells the markers seemed to be the result of several modifications. Of more interest were 3 cells (8.8%) with pulverized chromosomes, changes which are more often observed in cells infected by HSV (44). The 620-2T cells showed D18 and/or C-group supernumeration but less often than did the parental cell line. The aneuploidy was extended to almost all the cells with a shift of the modal number from a hyperdiploid to a hypodiploid mode. These cells showed marker chromosomes in the same frequency as the parental cells, but very few polyploid and no pulverized metaphases were observed in this tumor derivative.

The 620-7 cell line had different features. Although the modal number was 44 chromosomes, the pseudo- and hypodiploidy were extended to 17 and 28% of the cells, respectively. Twenty % of the cells were polyploid, ranging from 74 to 91 chromosomes, and the structural changes were very limited. The 620-7T cells, with a modal number of 44, had 42% pseudodiploid cells and only 6% normal cells. As in the 620-2T line, we observed very few polyploid cells and, here again, a shift toward the hypodiploidy (39%). The structural changes were more frequently found in this cell line (14%) cells. A small acrocentric, which could be a B10q-, was found in 3 cells. In the others, the markers were mediocentric chromosomes with different sizes.

Detection of Viral DNA. 32P-Labeled HSV-DNA was reannealed with transformed cell DNA to test for the presence of viral DNA sequences. The kinetics of reassociation were compared with kinetics observed when the probe was reannealed to normal hamster DNA containing specific amounts of viral DNA. As control, Hep-2, calf thymus, and normal hamster DNA were tested for possible contamination of the probe with host DNA. The results shown in Chart 1 give clear evidence of the presence of viral DNA in transformed and tumor cell lines. Assuming all cells in a clone carry the same DNA sequences, the size (a) and the number of copies (n), of the viral DNA associated with test DNA were determined by fitting the experimental data obtained from DNA reassociation kinetics to the equation:

\[ \frac{D_t}{D_0} = \frac{1 - a}{1 + k (P_0 + nT_0)t} \]

by computer-aided nonlinear regression (15) where \( D_t / D_0 \) is the fraction of DNA remaining single stranded at time t (in sec) \( P_0 \) is the molar concentration of the DNA probe (in mol of viral DNA per liter), \( T_0 \) is the molar concentration of the test DNA (in mol of the test DNA per liter), and k is the molar reassociation rate constant (liter/sec/mol of DNA). The clone 620-2 (passage 40) contains 43% of the viral genome in 6 copies, and the clone 620-7 (passage 43) contains 39% in 4 copies. The 2 tumor-derived cell lines 620-2T (passage 11) and 620-7T (passage 10) contain 47 and 43% in 2 copies, respectively.

In situ Hybridization. In situ hybridization was performed on uninfected and infected HEF by 1 PFU/cell of HSV-2 for 12 hr and also on transformed and tumor cell lines. The cell DNA was not denatured, so only viral RNA could be detected. As shown in Fig. 2b, the infected cells were very highly labeled in the cytoplasm as well as in the nucleus. When compared to controls (Fig. 2a), the transformed and tumor-derived cells (Fig. 2, c to f) showed significantly higher labeling, either in the cytoplasm or in the nucleus. Although the labeling seemed to be relatively homogeneous in the cytoplasm, nuclei in the same histological preparation had different labeling levels. In all clones, we observed highly labeled nuclei (more than 50 grains/nucleus) randomly distributed on the slide. They accounted for 80% of the nuclei in 620-2, 10% in 620-2T, 8% in 620-7, and 10% in 620-7T.

Immunofluorescence. The results obtained in the viral nucleic acids investigation led us to look for viral proteins. Transformed and tumor-derived lines, HSV-2, and infected and uninfected HEF were examined by indirect immunofluorescence.
Fig. 1. Karyotype with 45 chromosomes established from a cell of the 620-2 hyperdiploid subclone. The D-trisomy was classified in D18 pair on the basis of the R-banding technique.
Oncogenic Transformation by HSV

Table 1
Summary of the data in karyotype, immunofluorescence, in situ, and molecular hybridization analyses

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Total no. of cells</th>
<th>% of normal</th>
<th>% of pseudodiploid</th>
<th>% of hypodiploid</th>
<th>% of polyploid</th>
<th>% of pulivered metaphases</th>
<th>% of highly labeled nucleolus (&gt;50 grains/nucleus)</th>
<th>Molecular hybridization</th>
</tr>
</thead>
<tbody>
<tr>
<td>620.2</td>
<td>34</td>
<td>8.8</td>
<td>5.8</td>
<td>11.7</td>
<td>47</td>
<td>17</td>
<td>8.8</td>
<td>80</td>
</tr>
<tr>
<td>620.2T</td>
<td>36</td>
<td>11</td>
<td>5</td>
<td>47</td>
<td>38</td>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>620.7</td>
<td>35</td>
<td>31.4</td>
<td>17.1</td>
<td>28.5</td>
<td>2.8</td>
<td>20</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>620.7T</td>
<td>33</td>
<td>6</td>
<td>41</td>
<td>39</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

Whereas the normal cells did not react with the HSV-2 antiserum (Fig. 3a), the transformed and tumor lines showed different percentages of cells with positive fluorescence located mainly in the cytoplasm (Fig. 3, c to f). Clones 620-2 and 620-7, tested at passage 35, had 35 and 45% positive cells, respectively. Derivatives 620-2T (passage 6) and 620-7T (passage 8) showed 60 and 75% positive cells, respectively. From these results, it may be concluded that all the tested lines also have viral antigens.

**Protein Analysis.** Since we had evidence of viral antigens in these clones, it was of interest to analyze the polypeptide profiles in an attempt to identify differences between the normal and transformed cells. Monolayers of normal and HSV-2-infected HEF and transformed cells, labeled with [35S]methionine, were used. Fig. 4 shows an autoradiograph of 5 to 12% SDS-polyacrylamide gradient gel. Reproducible differences were found between the control and transformed cells: a polypeptide with a molecular weight of 200 x 10^3 (designated 200 K polypeptide), was detected in smaller quantities or not at all in all the transformed cells tested; a polypeptide molecular weight of 84 x 10^3, never found in normal controls, was consistently observed in the 620-7 clone and its tumor derivative (620-7T), as well as in other clones, but was not always visible in the 620-2 and 620-2T cells; a third polypeptide, with a molecular weight of 42.5 x 10^3, appeared in the transformed and tumor cells. Neither the control cells nor Ads5-transformed 14 b cells (46) appear to synthesize the latter 2 polypeptides. Identical results have been obtained by analysis of the clones at different passages. We cannot conclude that these polypeptides are of viral origin since they were not immunoprecipitated by antisera against an HSV-2-infected cell lysate (although the cells showed positive immunofluorescence with this serum). On the other hand, they might be minor viral polypeptides or cell polypeptides induced by transformation. It is significant that this is the first report of direct observation of polypeptides induced by transformation with HSV-2.

**DISCUSSION**

The data summarized in Table 1 suggest the following remarks.

In the karyotype analysis, the clones 620-2 and 620-7 showed different chromosome profiles at the same passage. The modal number and the aneuploidy extent were quite different. Similar numerical and structural changes were reported for other transformed lines (6, 34) with the difference that the clones appear to synthesize the latter 2 polypeptides. Identical results have been obtained by analysis of the clones at different passages. We cannot conclude that these polypeptides are of viral origin since they were not immunoprecipitated by antiserum against an HSV-2-infected cell lysate (although the cells showed positive immunofluorescence with this serum). On the other hand, they might be minor viral polypeptides or cell polypeptides induced by transformation. It is significant that this is the first report of direct observation of polypeptides induced by transformation with HSV-2.

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The molecular hybridization performed on the transformed and tumor-derived cells demonstrated the presence of several copies of a 40% fragment of the HSV genome. This homogeneous viral DNA content in the 620-2 and 620-7 cells does not necessarily mean that an identical DNA sequence is carried by the 2 clones (15, 31). In contrast to the findings of Frenkel et al. (15), who reported a decrease in the viral DNA sequences from 16 to 9% in the 333-8-9 tumor-derived cells, the sequence complexity in the 620-2T and 620-7T cells did not seem to be reduced upon passage of the parental cell lines through animals. The cells still retained 40% of the viral sequences. The reduction of the sequence complexity in the tumor cells was explained as possibly due to immunological selective pressure by the development of neutralizing antibodies against cells carrying higher sequence complexity of HSV-2 DNA (15). This kind of selective pressure does not seem to have occurred in our cells. In the 620-2T and 620-7T cells, we observed a decrease in the number of HSV-DNA sequence copies maintained in the cells. The suppression of cells with a higher number of copies by immune response, although possible, is difficult to assess because the DNA sequences which would determine the nature of the HSV antigens in the transformed cells were shown to be retained in the tumor derivatives. However, if we can assume that the different viral copies may be randomly integrated in the different chromosome pairs, any deviation in the chromosome number would modify not only the cell DNA content but also the number of copies of the associated viral DNA. Thus, the observed reduction in copy numbers in tumor cell lines might be related to the chromosome modifications noted above, mainly the increase of hypodiploid cells. Further studies of the fate of viral DNA in transformed and tumor-derived cells at higher passages, in correlation with the karyotype analysis, will help in defining more precisely the aneuploidy effect, if any, on the viral DNA content.

All of our clones were found to transcribe viral RNA’s as detected by in situ hybridization. In contrast to the reported data (6), the viral DNA transcription does not seem to be affected by passage of the cells in animals. We observed in all the cell lines different percentages of highly labeled nuclei (more than 50 grains/nucleus). There was no evident correlation between the tumorigenicity and the percentages of these highly labeled nuclei. The differences in nuclei labeling could be interpreted as follows: (a) they might reflect a difference in the total amount of viral DNA in individual cells. The highly labeled nuclei would retain more copies than would the ones with poor labeling. Then, by transcription of several copies of the same viral DNA fragment, these nuclei would contain larger amounts of viral RNA’s and (b) they might be due to a cell cycle-dependent transcription of the viral DNA sequences; Flannery et al. (14) studied the cell cycle-dependent expression of the HSV antigens in a 333-8-9 tumor-derived cell line and found the maximum antigen expression before and after the maximum DNA synthesis; a similar situation with a maximum of viral RNA transcription at a specific phase of the cell cycle might be possible. The in situ hybridization applied to synchronized cells should help in elucidating the significance of this observation.

The investigation of the HSV antigens by indirect immunofluorescence showed that all the transformed and tumor cell lines bore HSV antigens. The percentages of positive cells in clones as well as their increase in the tumor-derived cells are in agreement with the reported data of Macnab (25) and slightly higher than those of Duff and Rapp (10, 11). The polypeptide analysis by SDS-polyacrylamide gel electrophoresis showed 3 reproducible differences between the control and transformed cells. Similar findings have not been previously reported, and indeed, no polypeptide differences have been found in either biochemically or oncogenically HSV-transformed cells (199). These differences concern the following polypeptides: (a) the polypeptide with a molecular weight of 200,000 was found to be synthesized in small amounts, or not at all, in the transformed and tumor cell lines. It has been reported that a major external cell protein in normal fibroblasts was absent or much reduced in hamster cells transformed by sarcoma or polyoma virus (18) (it was named large external transformation-sensitive protein); the absence or reduction in large external transformation-sensitive protein synthesis seems also to occur in HSV-transformed cells; (b) polypeptide with a molecular weight of 84,000 was observed in the 620-7 and the 620-7T cells profile but not in the 620-2 and 620-2T cells line. There is no corresponding polypeptide in the normal HEF, but a small amount of this polypeptide could be seen in the HSV-2 infected cells. Although this polypeptide is not immunoprecipitated by serum raised against HSV-2-infected cells, it might be a minor viral polypeptide or a host protein induced by transformation; and (c) a polypeptide with a molecular weight of 42.5, was present in the transformed cells and their tumor derivatives but not in the control HEF; this polypeptide migrated close to the HSV thymidine kinase (4, 5, 16, 17, 20, 30, 35, 41, 43). Additional work is necessary for the characterization of this polypeptide with a molecular weight of 42,500 and also to prove that it is, in fact, virus related. From this study, it was also seen that the inoculation of the transformed cells in animals did not change their polypeptide profile; moreover, identical findings have been observed by analysis of several passages of the 620-2 and 620-7 cell lines and their tumor derivatives. At present, experiments are in progress to further study these observations and to define the functions of these polypeptides.

In conclusion, we have shown by analysis of DNA, RNA, and polypeptides that our lines 620-2 and 620-7 do not lose viral sequence complexity or expression following passage in animals. Thus, these lines appear ideally suited for more detailed studies of the role of HSV genes in transformation and oncogenicity.

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REFERENCES

5. Cheng, Y. C., and Ostrander, M. Deoxothymidine kinase induced in Hela...


Fig. 2. In situ hybridization with HSV-2 DNA labeled by nick translation with [3H]deoxyribonucleoside triphosphates. (a), normal HEF; (b), HEF infected by 1 PFU/cell of HSV-2 for 12 hr; (c and d), 620-2 and 620-2T cells, respectively; (e and f), 620-7 and 620-7T cells, respectively. × 100.
Fig. 3. HSV-specific immunofluorescence detected by total anti-HSV-2 antiserum. (a), Normal HEF; (b), plaque of HSV-2-infected HEF; (c and d), 620-2 and 620-2T cells, respectively; (e and f), 620-7 and 620-7T cells, respectively; a × 40 objective was used for all cells except (b) photographed with a × 10 objective.
Fig. 4. Polypeptide profiles from control and HSV-2 infected and transformed HEF. Normal, HSV-2-infected HEF and the transformed cells were labeled for 20 hr with $^{[35]}$S)methionine. Whole cell extracts were made for analysis by SDS discontinuous 5 to 12.5% gradient polyacrylamide gels. Equal amounts of radioactivity were applied to each track. The autoradiograph were developed after 13 days. The tracks are identified as follows: 7 and 7T for 620-7 and 620-TT cells, respectively; 2 and 2T for 620-2 and 620-2T, respectively; HEF for normal HEF; and HSV for HSV-2-infected HEF.
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