Integration and Transcription of Virus DNA in Herpes Simplex Virus Transformed Cell Lines

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SUMMARY

The physical state of the HSV 1 DNA present in two biochemically transformed cell lines, a revertant line and a supertransformed cell line, was determined. These cells all contained fragments of the HSV genome and the transformed and supertransformed cell lines expressed virus thymidine kinase. It was found that the virus DNA in these cells was maintained in a complex state with approximately half of the HSV DNA present in a covalently integrated state and the other half in a non-integrated state. There was no major cell line difference in the distribution of integrated and non-integrated virus DNA. RNA transcripts representing 5% of the HSV 1 genome are present in each of these lines. This is more than is required to code for the virus thymidine kinase present in the transformed and supertransformed cell lines and suggests the presence of other virus proteins in these cells.

INTRODUCTION

Murine cells lacking the enzyme thymidine kinase (tk-) have been transformed to a tk+ phenotype by u.v.-irradiated herpes simplex virus types 1 and 2 (HSV 1 and HSV 2; Munyon et al. 1971; Davis et al. 1974). The thymidine kinase expressed in these cells was of virus origin as shown by electrophoretic mobility, thermolability and serological specificity (Munyon et al. 1972; Davis et al. 1974; Thouless et al. 1976). The levels of thymidine kinase in these cells did not increase and decrease with the cell cycle as does the cellular thymidine kinase (Lin & Munyon, 1974) and the virus thymidine kinase was inducible by superinfection with tk- HSV (Lin & Munyon, 1974; Kit & Dubbs, 1977). A protein synthesized early after infection by HSV is necessary for this induction to take place (Leiden et al. 1976).

Solution hybridization experiments demonstrated that these biochemically transformed cell lines contain fragments of the HSV genome ranging from 3% to 20% and were present in single and multiple copies (Kraiselburd et al. 1975; Davis & Kingsbury, 1976; Sugino et al. 1977). Revertants have been selected from biochemically transformed cell line 139 by treatment with bromodeoxyuridine (BrdUrd) or 3H-thymidine, and some of these lines have been supertransformed by infection with u.v.-irradiated HSV (Chadha et al. 1977). Most revertant cell lines contained no detectable virus DNA; an exception was line 139 BrdUrd-2. The supertransformed lines contained virus DNA fragments representing 14% to 28% of the genome (Sugino et al. 1977).

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Cell fusion experiments indicated that the virus DNA fragment coding for thymidine kinase in biochemically transformed human cells is associated with two specific chromosomes (Donner et al. 1977). This suggests that the virus DNA may be present in an integrated state. It is known that HSV DNA can be found associated with the cellular genome after infection of permissive and non-permissive cells (Biegeleisen & Rush, 1976; Padgett et al. 1978).

The experiments presented here focus on two different aspects of the virus DNA present in biochemically transformed cells. First, the physical state of the virus DNA in two biochemically transformed cell lines, a revertant and a supertransformed cell line, has been determined. Experiments using the network technique and alkaline sucrose gradients indicated that the virus DNA in these cell lines was present in a covalently integrated state as well as a non-integrated state. Second, the fraction of the HSV 1 genome that is represented by RNA transcripts in these cell lines was determined. DNA–RNA solution hybridizations indicated that approx. 5% of the virus genome was transcribed in all the cell lines regardless of the size of virus DNA fragment present in the cell.

METHODS

Cells and viruses. Table 1 lists the designations and properties of the HSV 1-transformed, revertant and supertransformed cell lines used in this study. The properties of these cells and the derivation of the parental cell lines have been described elsewhere (Munyon et al. 1971; Chadha et al. 1977). The media used for propagation of each cell type have been described (Davis & Kingsbury, 1976). The revertant clone, r39 BrdUrd-2, was maintained in media containing 30 µg/ml BrdUrd. NHF cells are normal human foreskin fibroblasts originating in this laboratory. HSV 1 hybridization probes were prepared from virions of the JH strain of HSV 1. This strain shows complete homology to the KOS strain used in the transformation experiments (Sugino & Kingsbury, 1976).

Extraction of high molecular weight cellular DNA and the preparation of hybridization probes. High mol. wt. DNA was extracted from cell monolayers using a phenol extraction as described earlier (Davis & Kingsbury, 1976) with modifications in the technique to avoid shearing forces whenever possible. 6H-thymidine labelled HSV DNA for use as a hybridization probe was prepared as described earlier (Davis & Kingsbury, 1976). The HSV 1 strain JH probes used in these studies had a specific activity of either 6-6 × 10⁶ or 2-5 × 10⁶ ct/min/µg. All DNA used in hybridization reactions was sheared at 0 to 2 °C for 2 min with a Branson sonifier fitted with a micro tip. This treatment gave fragment lengths of 350 nucleotides as determined by sedimentation in alkaline sucrose gradients.

Extraction of cellular RNA. Cell pellets were resuspended in 5 vol. of 0-2 M-NaCl and 1 mM-EDTA and lysed by the addition of SDS to 1%. The lysate was sheared in a Waring blender and incubated with 50 µg/ml of Proteinase K at 37 °C for 1 h. This suspension was extracted with phenol, phenol–chloroform and chloroform, and the RNA was precipitated by the addition of cold ethanol, storage overnight at −20 °C and centrifugation at 4000 g. The RNA was resuspended in 50 mM-MgCl₂ and 10 mM-tris, pH 7-5, and incubated for 1 h with 50 µg/ml pancreatic DNase. This was followed by the addition of Proteinase K at 50 µg/ml and incubation for 1 h. The solution was again extracted with phenol and chloroform, and then precipitated by the addition of cold ethanol as above. The RNA pellet was resuspended in 1 mM-EDTA, the absorbance at 260 and 280 nm measured and the RNA stored at −70 °C. All steps were carried out using sterile solutions and glassware.

Hybridization conditions. All DNA–DNA solution hybridizations were carried out as described earlier (Sugino et al. 1977) except that the DNA in the reactions was denatured by
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the addition of NaOH to 100 mM before addition of the sodium phosphate reaction buffer. In every case the concentration of unlabelled 'driver' DNA was adjusted to provide an excess of cellular DNA to give a ratio of 50 to 100 cellular genomes for each virus probe genome.

Hybridizations with cellular RNA were carried out in 200 μl vol. Approx. 2 × 10^-3 μg of 3H-HSV DNA probe was denatured by the addition of NaOH to 100 mM and incubation for 10 min at room temperature. PB (sodium phosphate buffer containing equal molar concentrations of monobasic and dibasic sodium phosphate, pH 6.8) was added to 0.48 M final concentration and cellular RNA was added to a concentration of 1.7 to 3.6 mg/ml. A zero time sample was removed and the reactions were covered with mineral oil and incubated at 70 °C. All time points taken during the reaction were immediately diluted into 0.12 M-PB containing 0.2% SDS. The single and double strands were separated on a hydroxyapatite column. Single strands were eluted with 0.12 M-PB with 0.2% SDS and double strands were eluted with 0.3 M-PB. The amount of reassociation was calculated by determining the percentage of counts in the sample that were in a double-stranded form at each time point.

The results of the hybridizations were plotted using the equation:

\[
\frac{I}{I_{fsS_{0}}} = KC_{0}t + 1,
\]

where \(I/fss_{0}\), the fraction of probe remaining single stranded at time \(t\), equals \(C_{0}/C\) (Britten & Kohne, 1968). By plotting \(I/fss\) against \(C_{0}t\) the slope of the line is equal to \(K\). The increase in reaction rate caused by the presence of virus sequences in transformed cell DNA results in an increase in the slope of the plot. By comparing the slope of a reaction containing transformed cell DNA to the slope of a reaction containing a known amount of HSV DNA (self reaction) the amount of virus DNA present in transformed cell DNA, or fractions thereof, can be calculated (Gelb et al. 1971). The curves of the reactions which contain transformed cell DNA are not linear throughout the reaction because only a fragment of the virus genome is present in the transformed cell (Davis & Kingsbury, 1976; Sugino et al. 1977). Therefore the initial slope, corresponding to the driven part of the reaction, was used for quantification of the amount of virus DNA present in all cases.

Network analysis. The formation of DNA networks of high mol. wt. cellular DNA was similar to the method described by Bellett (1975). High mol. wt. DNA was diluted to 1.25 mg/ml and denatured by the addition of NaOH to 100 mM. PB was added to 0.48 M and the solution reannealed to a Cot of 100 mol. s/l at 60 °C. The solution was diluted twofold in 0.48 M-PB at 60 °C and centrifuged in a SW50.1 rotor for 20 min at 35000 rev/min at 10 °C. The supernatant was removed, dialysed against distilled water and lyophilized before suspension in 1 mM-EDTA and shearing. The network pellet was resuspended directly into 1 mM-EDTA and sheared.

The amount of cellular DNA contained in the network and supernatant fractions was determined by measuring the A260 of the two fractions. The amount of HSV DNA in each fraction was determined by DNA–DNA solution hybridizations as described above. The network experiments included a trapping control consisting of a sample of the networking reaction to which unsheared 3H-HSV DNA was added. After the network procedure, the supernatant and network of the control reaction were placed in scintillation vials and counted. The percentage of counts found in the network was considered the trapping value and was used to correct the data as presented in Results.

Alkaline sucrose gradients. Alkaline sucrose gradients (5 to 20%), containing 0.03 M-tris, 0.005 M-EDTA, 1.0 M-NaCl and 0.3 M-NaOH, were formed in SW27 tubes. Five × 10⁶ cells were layered on a gradient followed by 1 ml of lysis buffer containing 0.5 M-NaOH and
Table 1. Cell lines and their properties

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Type</th>
<th>% genome</th>
<th>Copies/haploid genome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>139</td>
<td>Transformed, tk⁺</td>
<td>9</td>
<td>2.5†</td>
<td>W. Munyon</td>
</tr>
<tr>
<td>171</td>
<td>Transformed, tk⁺</td>
<td>17</td>
<td>1.5</td>
<td>W. Munyon</td>
</tr>
<tr>
<td>139 Brd Urd-2</td>
<td>Revertant, tk⁻</td>
<td>9</td>
<td>1.0</td>
<td>K. Chadha</td>
</tr>
<tr>
<td>139 R12S3</td>
<td>Supertransformed, tk⁺</td>
<td>28</td>
<td>3.0</td>
<td>K. Chadha</td>
</tr>
</tbody>
</table>

* Sugino et al. (1977).
† Davis & Kingsbury (1976).

RESULTS

Network analysis of integrated virus DNA

The network technique, as described by Varmus et al. (1973) and Bellett (1975), was used to determine the physical state of the HSV 1 DNA contained in two biochemically transformed cell lines, a revertant of one of these cell lines and a supertransformed cell line (Table 1). High mol. wt. DNA from these cells was alkali denatured and reassociated to a Cₒₒ of 100 mol. s/l. At this Cₒₒ the repetitive sequences present in the cellular DNA reassociated forming a network containing 66 to 85 % of the DNA present in the reaction. This network, which should contain any integrated virus DNA, was separated from the supernatant by centrifugation as described in Methods.

For each network experiment, five quantitative hybridizations with high sp. act. ³H-HSV DNA were carried out. The network and supernatant fractions from a networking reaction were hybridized as was an untreated sample of the same DNA. In addition, a self reaction, containing only ³H-HSV probe and DNA known to be free of HSV sequences, and a reconstruction reaction, containing a known amount of virus DNA, were run at the same time.

The reaction kinetics of all four networking experiments presented in Fig. 1 to 4 indicate that there was virus DNA present in both the network and supernatant fractions of the DNA from each cell line. In order to keep the ratio of cellular to probe DNA high enough to ensure maximum sensitivity variable fractions of the network supernatant and pellet were used. In Fig. 1 to 4 the apparent rate of hybridization of the network supernatant is very high; however, this fraction constitutes the supernatant from the entire networking reaction uncorrected for the total number of cells from which it was obtained. The hybridization to the networking pellet was done on approx. 25 % of the entire sample, so that...
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Fig. 1. Kinetics of hybridization of \(^{3}H\)-HSV DNA with DNA from a network reaction of 139 cell DNA. The reassociation of \(1.8 \times 10^{-3} \mu g\) of \(^{3}H\)-HSV DNA (sp. act. \(6.6 \times 10^6 \text{ ct/min/\(\mu g\)}\)) was determined in the presence of 450 \(\mu g\) of one of the following: 139 DNA (\(\square\)), 139 network DNA (\(\blacktriangle\)), 139 supernatant DNA (\(\bigcirc\)), Balb/C DNA (\(\Delta\)) and Balb/C DNA plus \(5.4 \times 10^{-3} \mu g\) of HSV DNA (\(\bigcirc\)) in a total reaction of 500 \(\mu l\). The \(C_\alpha t\) values have been corrected for salt concentration and represent \(C_\alpha t\) equivalents (E \(C_\alpha t\)) in \(0.12 M\)-PB.

Fig. 2. Kinetics of hybridization of \(^{3}H\)-HSV DNA from a network reaction of 171 cell DNA. The reassociation of \(1.8 \times 10^{-3} \mu g\) of \(^{3}H\)-HSV DNA (sp. act. \(6.6 \times 10^6 \text{ ct/min/\(\mu g\)}\)) was determined in the presence of 450 \(\mu g\) of one of the following: 171 DNA (\(\square\)), 171 network DNA (\(\bigcirc\)), 171 supernatant DNA (\(\bigcirc\)), Balb/C DNA (\(\Delta\)) and Balb/C DNA plus \(5.4 \times 10^{-3} \mu g\) of HSV DNA (\(\bigcirc\)) in a total reaction of 500 \(\mu l\). The \(C_\alpha t\) values have been corrected for salt concentration and represent \(C_\alpha t\) equivalents in \(0.12 M\)-PB.

Fig. 3. Kinetics of hybridization of \(^{3}H\)-HSV DNA with DNA from a network reaction of 139 BrdUrd-2 cell DNA. The reassociation of \(1.8 \times 10^{-3} \mu g\) of \(^{3}H\)-HSV DNA (sp. act. \(6.6 \times 10^6 \text{ ct/min/\(\mu g\)}\)) was determined in the presence of 450 \(\mu g\) of one of the following: 139 BrdUrd-2 DNA (\(\square\)), 139 BrdUrd-2 network DNA (\(\star\)), 139 BrdUrd-2 supernatant DNA (\(\bigcirc\)), Balb/C DNA (\(\Delta\)) and Balb/C DNA plus \(7.2 \times 10^{-3} \mu g\) of HSV DNA (\(\bigcirc\)) in a total reaction of 500 \(\mu l\). The \(C_\alpha t\) values have been corrected for salt concentration and represent \(C_\alpha t\) equivalents in \(0.12 M\)-PB.

Fig. 4. Kinetics of hybridization of \(^{3}H\)-HSV DNA with DNA from a network reaction of 139 RI-253 cell DNA. The reassociation of \(1.8 \times 10^{-3} \mu g\) of \(^{3}H\)-HSV DNA (sp. act. \(6.6 \times 10^6 \text{ ct/min/\(\mu g\)}\)) was determined in the presence of 450 \(\mu g\) of one of the following: 139 RI-253 DNA (\(\square\)), 139 RI-253 network DNA (\(\star\)), 139 RI-253 supernatant DNA (\(\bigcirc\)), human placenta DNA (\(\bigcirc\)) and human placenta DNA plus \(5.4 \times 10^{-3} \mu g\) of HSV DNA (\(\bigcirc\)) in a total reaction of 500 \(\mu l\). The \(C_\alpha t\) values have been corrected for salt concentration and represent \(C_\alpha t\) equivalents in \(0.12 M\)-PB.
Table 2. Amount of integration of HSV DNA in transformed, revertant and supertransformed cell lines

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell line</th>
<th>% virus DNA integrated</th>
<th>% total HSV DNA recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemically transformed</td>
<td>139</td>
<td>50*</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>139 (alkaline sucrose)</td>
<td>43</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>171</td>
<td>62</td>
<td>110</td>
</tr>
<tr>
<td>Revertant</td>
<td>139 BrdUrd-2</td>
<td>45</td>
<td>115</td>
</tr>
<tr>
<td>Supertransformed</td>
<td>139 R12S3</td>
<td>61</td>
<td>120</td>
</tr>
</tbody>
</table>

* The % virus DNA integrated was calculated from the equation of Bellett (1975) which states:

\[ I = \frac{Vn-Cn}{1-(Ds+Cn)} \]

where \( I \) is the fraction of the virus DNA integrated; \( Vn \) is the fraction of the virus DNA found in the network fraction; \( Ds \) is the fraction of the cellular DNA in the supernatant; and \( Cn \) is the fraction of trapping in the control network. The total recovery of virus DNA is a comparison of the total amount recovered in each fraction divided by the amount of virus DNA in an equivalent sample.

several correction factors must be applied. Calculations to quantify the amount of virus DNA present in an integrated and non-integrated state were done in three parts. First, the amount of virus DNA present in each hybridization reaction was calculated by comparing the initial rate of the reactions to the initial rate of the self reaction. The reconstruction reaction was used to ensure that the hybridizations and calculations were accurate. Second, the amount of virus DNA detected in the network and supernatant fractions was corrected to account for the relative fractional amount of the original DNA present in each fraction.

In order to be able to compare experiments two other factors must be taken into account when calculating the amount of integration of virus DNA. These factors are the amount of trapping of non-integrated sequences into the network and the amount of cellular DNA that actually enters the network. To correct for these factors an equation derived by Bellett (1975) was used to calculate the amount of virus DNA integrated into the cellular genome in each case. In all experiments the amount of trapping was below 5%. The results of multiple experiments analysed by this method are contained in Table 2. In all cases approx. 50% of the virus DNA was associated with the cellular DNA and 50% was present in a non-integrated state. All network reactions reported were done using alkaline denaturation of cellular DNA before the networking reaction in contrast to the heat denaturation used by Varmus et al. (1973) and in part by Bellett (1975). Thus, the association of virus DNA to cellular sequences must be by covalent linkages.

Alkaline sucrose gradient analysis of integrated virus DNA

To ensure that the results from the network experiments were not due to some undefined aspect of HSV integration a second technique was used to analyse the integration of HSV DNA in cell line 139. The alkaline sucrose gradient analysis used is based on the technique of Varmus et al. (1976). Five × 10⁶ 139 cells were layered on alkaline sucrose gradients and lysed by the addition of 0.5 M-NaOH followed by incubation overnight at 0 °C before centrifugation. This results in a very gentle extraction of the DNA present in the cells because no shearing forces are involved; hence the cellular DNA remains in a very high mol. wt. form which can be separated from smaller non-integrated virus sequences. Any covalently integrated virus DNA should remain attached to the high mol. wt. cellular DNA during centrifugation and be found associated with this DNA in the bottom fractions of the gradient.
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Fig. 5. A 5 to 20% alkaline sucrose gradient was layered with 3H-HSV DNA and 5 x 10^6 139 cells grown in the presence of 32P. After addition of lysing buffer and incubation at 0°C for 18 h, the gradient was centrifuged for 4 h in a SW27 rotor at 27,000 rev/min, collected and the acid-precipitable radioactivity counted in each fraction. ---, 32P-labelled 139 DNA; ---, 3H-labelled HSV DNA. Thirty-three gradients were layered with 5 x 10^6 139 cells, lysed and centrifuged as above. The top, middle and bottom fractions were collected and pooled, and the DNA concentrated from each as discussed in Methods. The DNA from each fraction was hybridized with 3H-HSV DNA and the distribution of virus DNA in the gradients is represented by the bars superimposed on the gradient profile.

The profile of a control gradient containing 32P-labelled 139 cells and 3H-HSV DNA shows that the high mol. wt. cellular DNA sediments rapidly to the bottom of the gradient while the added virus DNA remains in the top fractions (Fig. 5). The 32P peak at the top of the gradient consisted of acid-insoluble counts. This material which absorbs very little at 260 nm has been observed by others and most likely is composed of phospholipids and phosphoproteins although it may contain a small amount of mitochondrial DNA. This control gradient indicates that with the amount of cells lysed on the gradients no trapping of non-integrated DNA takes place.

Multiple gradients with unlabelled 139 cells were centrifuged and collected into top, middle and bottom fractions. The DNA was extracted from each of these fractions and the amount of virus DNA present in each fraction was determined by hybridization to the 3H-HSV probe. The percentage of the virus DNA present in 139 cells found in each fraction is represented by the bars superimposed on the profile of a control gradient in Fig. 5. The hybridizations indicate that 43% of the virus DNA in 139 cells is associated with high mol. wt. cellular sequences while 32% is non-integrated and remains in the top fractions. The 25% of the virus DNA which was found in the middle fraction could have been due to mixing of the gradients during collection, preferential integration of some virus sequences into small cellular sequences, or rapid sedimentation of a non-integrated supercoiled circular form of the virus DNA.

These results, which show that 43% of the virus DNA was integrated in 139 cells, are very close to the results from the networking experiments. This is an indication that the two techniques are measuring the same physical parameter and that the network analysis is as accurate as alkaline sucrose gradients for the determination of the physical state of virus DNA.
Detection of virus RNA transcripts

Total cellular RNA was extracted from 139, 139 BrdUrd-2, 139 R12S3 and 171 cells, and hybridized with a small amount of 3H-HSV DNA as described in Methods. The kinetics of reaction are shown in Fig. 6 for RNA from 139 and 139 BrdUrd-2 cells and in Fig. 7 for RNA from 171 and 139 R12S3 cells. The reactions shown are typical. Because of the small amount of reaction the hybridizations were repeated five to eight times to ensure an accurate estimate of the extent of reaction. In addition several RNA concentrations were examined in order to obtain a true plateau value. The range of values and the average extent of reaction for RNA from each cell line is presented in Table 3. In all cases the reassociation of a self-reaction containing NHF RNA was subtracted from the experimental values. In each case, the plateau of the reaction was reached by an RNA Cøt of 500 during which time the probe self-reaction was less than 1%.

The results indicate that each cell line contains approximately the same fraction of the HSV genome transcribed into RNA. In the case of 139 and 139 BrdUrd-2, 5% of the genome represented as RNA could be the total coding capacity of the virus DNA fragment present in the cells, assuming transcription of only one strand of the virus DNA. Cell lines 171 and 139 R12S3 contain larger fragments of the virus genome and could theoretically have larger amounts of virus RNA transcribed. The results here indicate that there was a very similar amount of the virus genome present as RNA transcripts in every cell line and that the level of transcription is independent of virus DNA fragment size.

Table 3. Percentage of the HSV 1 genome transcribed in biochemically transformed, revertant and supertransformed cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>% HSV-1 genome transcribed (range)</th>
<th>% of HSV-1 virus fragment transcribed</th>
</tr>
</thead>
<tbody>
<tr>
<td>139</td>
<td>5 (2-8)</td>
<td>55</td>
</tr>
<tr>
<td>171</td>
<td>5 (3-9)</td>
<td>29</td>
</tr>
<tr>
<td>139 BrdUrd-2</td>
<td>4 (1-8)</td>
<td>46</td>
</tr>
<tr>
<td>139 R12S3</td>
<td>5 (2-11)</td>
<td>18</td>
</tr>
</tbody>
</table>

Detection of virus RNA transcripts

Fig. 6. Kinetics of hybridization of 3H-HSV DNA with transformed cell RNA. The reassociation of 7·1 x 10^-9 µg of 3H-HSV DNA (sp. act. 2·5 x 10^6 ct/min/µg) was determined in the presence of 710 µg of 139 RNA (●) or 139 BrdUrd RNA (○) in a reaction volume of 200 µl. The self reaction has been subtracted from each time point. The Cøt values have been corrected for salt concentration and represent Cøt equivalents in 0·12 M-PB.

Fig. 7. Kinetics of hybridization of 3H-HSV DNA with transformed cell RNA. The reassociation of 7·1 x 10^-9 µg of 3H-HSV DNA (sp. act. 2·5 x 10^6 ct/min/µg) was determined in the presence of 710 µg of 171 RNA (□) or 139 R12S3 RNA (■) in a reaction volume of 200 µl. The self reaction has been subtracted from each time point. The Cøt values have been corrected for salt concentration and represent Cøt equivalents in 0·12 M-PB.
**DISCUSSION**

The maintenance of virus DNA in an integrated state in the genome of cells transformed by DNA tumour viruses has become a common phenomenon. Cells transformed by human and avian adenoviruses contain virus DNA present in a covalently integrated state (Doerfler, 1968; Bellett, 1975) as do cells transformed by SV40 (Sambrook et al. 1968; Botchan et al. 1976; Ketner & Kelly, 1976). Analysis of the physical state of the virus DNA present in cells transformed by herpesvirus has become a complex problem. Cells transformed by Epstein-Barr virus (EBV) can contain linear integrated virus sequences or non-integrated circular forms of the virus DNA. Some cell lines, most notably Raji, seem to contain both integrated and non-integrated forms of EBV DNA (Adams et al. 1973; Adams & Lindahl, 1975; Lindahl et al. 1976; Adams et al. 1977).

Integrated virus DNA is not present in transformed cells alone. Virus DNA can become integrated during lytic infections with SV40 (Hirai & Defendi, 1972), adenovirus (Burger & Doerfler, 1974; Tyndall et al. 1978), and HSV (Biegeleisen & Rush, 1976); likewise, HSV 1 DNA can become integrated within 16 h after infection of non-permissive XC cells (Padgett et al. 1978).

The experiments reported here demonstrate that cells biochemically transformed by HSV 1 contain integrated virus DNA. However, it was also demonstrated that, in every case, non-integrated virus DNA is also present. Approximately half of the virus DNA was in an integrated state and half in a non-integrated state in every cell line examined. Since all the procedures to assay integration used in these experiments employed alkali denaturation conditions, the integration measured must be due to covalently integrated virus sequences.

The two major techniques used in these experiments, network formation and alkaline sucrose density gradient centrifugation are known to measure the same physical parameter as a caesium chloride gradient, the other widely used technique for studying virus integration (Tyndall et al. 1978). Network formation and alkaline sucrose have the extra advantage of much less non-specific trapping of the virus DNA in the high mol. wt. cellular DNA. In all cases the cells were subjected equally to both procedures and the amount of agreement between them is remarkable.

Unlike an earlier study of the integration of an HSV-1 derived gene (Pellicer et al. 1978) the cell lines studied here were transformed with u.v. inactivated virus particles and not a purified and defined sequence of the HSV genome. The lack of knowledge about the exact fragment present in these cells makes the use of some of the techniques reported earlier (Pellicer et al. 1978) for the study of integration very difficult.

Because of the small amounts of virus DNA present in the cell lines examined a quantitative analysis of the networking experiments, as described by Bellett (1975), was used. This analysis takes into account the amount of trapping of non-integrated virus sequences into the network and required a control networking to be carried out with each networking experiment.

In contrast to SV40 and adenovirus, where a large amount of information concerning the virus transcripts present in transformed cells is available, little is known of the virus RNA transcripts present in HSV transformed cells. Reports by Collard et al. (1973) and Jamieson et al. (1976) have established the presence of virus RNA in HSV transformed cells. Since cell lines biochemically transformed by HSV invariably contain only a fraction of the virus genome (Davis & Kingsbury, 1976; Sugino et al. 1977) RNA transcripts representing only a portion of the virus genome are expected.

The experiments reported here indicate that approx. 5% of the HSV genome was transcribed in every cell line examined. A transcript of this size indicates that the entire fragment of virus DNA present in 139 and 139 BrdUrd-2 cells is being transcribed assuming transcription of only one strand of DNA. Cell lines 171 and 139 R12S3 contained larger virus...
DNA fragments and could have RNA transcripts of a more extensive region of the genome. The results from these experiments, however, indicate that only a 5% fragment was transcribed in these cells also.

The limited amount of transcription detected in this case could be due to several factors. A portion of the transcripts could be present in very low concentrations and not be detectable or the RNA could be rapidly degraded. It is also possible that there is some type of transcriptional control operating on the virus DNA sequences in the cell.

Thymidine kinase, with a mol. wt. of 44,000 requires 0.85% of the coding capacity of the HSV genome (Wigler et al. 1977). The presence of RNA transcripts greatly exceeding this amount suggests the presence of other virus proteins in biochemically transformed cells. Initial observations made in this laboratory indicate that 60 to 75% of the virus RNA transcripts present in the biochemically transformed cell lines 139 and 139 R12S3 are homologous to the immediate early (alpha) RNA transcripts present during a permissive infection with HSV. Thus it is possible that proteins present early in productive infection with HSV are also present in transformed cells. It may be the presence of these proteins, associated primarily with virus replication that leads to what appears to be a dynamic process within the cell in contrast to the stable integration of the tk gene fragment reported previously (Pellicer et al. 1978).

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REFERENCES


HSV DNA in transformed cells


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