Fate of Virus DNA in the Abortive Infection of Human Lymphoid Cell Lines by Epstein–Barr Virus

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Human lymphoid cell lines have so far only been established from cells exposed to Epstein–Barr virus (EBV) (Henle et al. 1967). Such cell lines contain virus DNA in addition to cellular DNA (zur Hausen & Schulte-Holthausen, 1970), but the virus genome is present in a repressed state (Hampar et al. 1972). Some cell lines of this type can be superinfected with EBV. This results in an abortive infection, with the expression of several early virus functions (Henle et al. 1970; Gergely, Klein & Ernberg 1971) but no production of virus particles. In the present work, biologically active EBV, labelled with [3H]-thymidine, was partly purified from the culture medium of the cell line P3HR-1 which produces virus (Hinuma et al. 1967). The fate of the radioactive virus DNA was then investigated after abortive infection of two different human lymphoid cell lines.

Crude [3H]-thymidine-labelled EBV was prepared from 2 l. of cell culture medium by repeatedly centrifuging at high- and low-speed (Schulte-Holthausen & zur Hausen, 1970). The material was subsequently treated with pancreatic DNase (50 #g./ml., 2 hr, 21°), and the virus 'precipitated' with 8 % polyethylene glycol 6000 (McSharry & Benzinger, 1970), leaving fragmented cellular DNA in solution. The final virus preparation contained 65 % virus DNA (230,000 counts/min./7/~g.) and 35 % cellular DNA oligonucleotides (120,000 counts/min./< 5/~g.), as judged from CsCl equilibrium and sucrose gradient velocity sedimentation experiments. From the amount of virus DNA obtained, it was estimated that the preparation contained approx. 4 × 10¹⁰ virus particles, assuming a mol. wt of 10⁸ for this DNA (see below).

When the two lymphoid cell lines Raji and NC 37 were infected with [3H]-EBV (approx. 500 virus particles/cell), 3 to 15 % of the labelled DNA was adsorbed to the cells in different experiments. Of this material, approx. 35 % was retained by the cells 40 hr after infection (Table 1). At that time, 'early antigen' (Henle et al. 1970) was induced in 4 % of Raji cells infected with a 100-fold dilution of the same virus preparation (multiplicity of infection approx. 5). Experiments with comparable virus preparations in a series of dilutions indicate that 50 to 100 % 'early antigen' was induced after infection at the high multiplicity used.

The cells were lysed 40 hr after infection by incubation at a concentration of 10⁷ cells/ml. in 0.25 M sucrose, 0.01 M tris-HCl, pH 7.2, 0.001 M MgCl₂, 0.005 M CaCl₂, 0.5 % NP 40 at 0° for 10 min. At the end of this incubation, very few intact cells remained (< 1 %). After centrifuging at 1000 g for 7 min., the cloudy supernatant fluid ('cytoplasm extract') was removed by aspiration, and the pellet of cell nuclei suspended in 0.15 M NaCl, 0.01 M tris-HCl, pH 7.2, 0.001 M MgCl₂, 0.005 M CaCl₂, at 0° (2 × 10⁷ cell nuclei/ml.). In infected Raji cells, 60 ± 10 % of the total intracellular radioactivity was repeatedly found in the nuclei, and 40 % in the cytoplasm (Table 1). In infected NC 37 cells, 40 ± 10 % of the radioactive material was found in the nuclei. When nuclei from infected cells were disrupted by brief sonic irradiation and subsequently treated with pancreatic DNase, 50 to 80 % of the radioactive material was converted to an acid-soluble form. Treatment of intact virus particles in the same fashion did not lead to solubilization of virus DNA. As at least 90 % of the
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Fig. 1

Fig. 1. Size of the EBV DNA present in cell nuclei 40 hr after infection. The direction of sedimentation is from right to left. Arrow indicates the position of [32P]-PM2 DNA.

Fig. 2. Density of the EBV DNA present in cell nuclei 40 hr after infection. ■ = ■, E[sub]260; ○ = ○ [3H]-EBV DNA; ▲ = ▲ [32P] Escherichia coli DNA.

Table 1. Uptake of [3H]-EBV (2 x 10[10] virus particles)
by Raji cells (4 x 10[7] cells)

<table>
<thead>
<tr>
<th>Labelled material added to cells (65 % EBV DNA)</th>
<th>[3H], % of counts/min.</th>
<th>% of input</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material adsorbed to cells</td>
<td>180,000</td>
<td>100</td>
</tr>
<tr>
<td>Material released into the medium during incubation for 40 hr</td>
<td>11,000</td>
<td>6·1</td>
</tr>
<tr>
<td>Material retained by cells (&gt; 95 % EBV DNA)</td>
<td>7,000</td>
<td>3·9</td>
</tr>
<tr>
<td>In cytoplasm</td>
<td>4,000</td>
<td>2·2</td>
</tr>
<tr>
<td>In nuclei</td>
<td>1,700</td>
<td>0·9</td>
</tr>
<tr>
<td></td>
<td>2,300</td>
<td>1·3</td>
</tr>
</tbody>
</table>

labelled DNA remaining in the cell nuclei 40 hr after infection was virus DNA (Fig. 1, 2), it is concluded that > 50 % of this DNA was present in a DNase-sensitive form.

Nuclei from infected cells were lysed by addition of 1 vol. of 0·1 M-tris-HCl, pH 9·0, 0·02 M-EDTA, 1 % Sarcosyl NL97, and 0·5 volume of pronase (5 mg./ml. in 0·05 M-tris-HCl, pH 7·5, pre-incubated for 2 hr at 37). A part of the extracted DNA (5 #g.) was centrifuged on a linear (5 to 20 %) sucrose gradient containing 2 M-NaCl, 0·01 M tris-HCl, 0·01 M EDTA, pH 9 for 5 hr at 25,000 rev./min. in a Spinco SW27 rotor at 20°. [32P]-PM2 DNA was added as internal marker. As a control, nuclei from uninfected cells, uniformly labelled with [3H]-thymidine, were extracted by the same procedure. The properties of this cellular DNA were typical of high-mol. wt DNA obtained from chromosomes by gentle extraction. Its sedimentation coefficient (73 s) corresponded to an average mol. wt of the DNA of 160 x 10[6] (Freifelder, 1970). When nuclei from non-labelled cells infected with radioactive EBV were centrifuged under identical conditions, > 90 % of the radioactive DNA component instead sedimented as a sharp peak at 59 s, i.e. as an apparently homogenous DNA molecule of a mol. wt of 100 x 10[6] (Fig. 1). In separate experiments, DNA from isolated EBV particles was also found to have a sedimentation coefficient of 59 ± 2 s on co-sedimentation with phage T4 DNA or phage PM2 DNA.

The high mol. wt DNA from nuclei from infected cells was also fractionated by sedimentation to equilibrium in CsCl density gradients. The radioactive DNA banded at a density corresponding to a base composition of 58 % guanine-cytosine base pairs in both
neutral and alkaline gradients. This is also the density of DNA isolated from EBV particles. In contrast, the great majority of the DNA from nuclei from infected cells, which was cellular in origin, was of a different density. The same result was obtained when growing Raji or NC 37 cells were infected, or when the cells had been pre-treated with $1.6 \times 10^{-5}$M bromodeoxyuridine for 3 days and then infected with EBV in medium containing $1.2 \times 10^{-5}$M arabinosyl-cytosine to amplify the density difference between virus and cellular DNA, and to prevent DNA replication. An experiment of the latter type is shown in Fig. 2. The concentration of arabinosyl-cytosine employed inhibits DNA synthesis but does not interfere with the expression of early virus functions on superinfection in this system (Gergely et al. 1971). Part of the high mol. wt DNA (15 µg.) recovered from nuclei 40 hr after infection was centrifuged in CsCl containing 0.01M tris HCl, 0.001M EDTA, pH 8.5 for 65 hr at 30,000 rev./min. in a Spinco SW 50.1 rotor at 20°. Radioactive material banding at a position intermediate between cellular DNA and free virus DNA was not observed in any of these experiments. The data therefore lend no support to the concept of covalent association between virus DNA and cellular DNA, and integration of virus DNA at the scale observed in certain other systems, e.g. in the abortive infection of baby hamster kidney cells by adenovirus 12 (Doerfler, 1968), clearly did not occur.

The uncoated EBV DNA in the cell nuclei remained in a high mol. wt form 40 hr after infection. Incoming foreign DNA has been reported to be similarly preserved in a number of other cases. On infection of KB cells with adenovirus 5, virus DNA re-isolated from cell nuclei 18 hr after infection had the same size as free virus DNA (Sussenbach, 1971). Further, on infection of human or mouse embryonic cells by polyoma pseudo-virus, the incoming DNA was found in the cell nuclei 24 hr after infection in an uncoated, macromolecular form, but integration did not occur to a detectable extent (Qasba & Aposhian, 1971). As super-infecting EBV DNA is found in the cell nuclei, and is retained there with no decrease in mol. wt for at least 40 hr, the observed expression of early virus functions 20 to 50 hr after superinfection (Henle et al. 1970; Gergely et al. 1971) may depend on transcription of the incoming DNA.

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