Synthesis of Herpes Simplex Virus DNA in Preparations of Chromatin from Infected Cell Nuclei

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SUMMARY

Chromatin prepared from cells infected with Herpes simplex virus type 1 or type 2 can synthesize both virus and cell DNA in vitro. The rate of synthesis is comparable to that of isolated whole nuclei. Incorporation is limited, and both cell and virus DNA synthesis are sensitive to the presence of virus-specific antiserum and phosphonoacetate. In chromatin from cells infected with a phosphonoacetate resistant virus mutant, both types of DNA synthesis are resistant to the presence of the inhibitor.

Herpes simplex virus (HSV) DNA synthesis has been shown to take place in nuclei isolated from infected cells by a number of workers (Biswal & Murray, 1974; Becker & Asher, 1975; Bolden et al., 1975; Kolber, 1975). Recently a system has been described (Francke, 1977a, b) in which lysates of HSV-1 infected cells, in addition to nuclei, were found to be capable of the synthesis of virus DNA whose general properties approach that of material synthesized in vivo. Observations in the light microscope and with the electron microscope using autoradiography of thin sections from infected serum-starved cells (F. J. Rixon, M. A. Atkinson & J. Hay, unpublished data) suggested some association of labelled virus DNA with chromatin during virus replication. Thus, we undertook the preparation of chromatin from HSV-infected cells as a possible first step in the recognition of an HSV DNA synthesizing complex.

BHK 21/C13 cells were mock infected or infected at an m.o.i. of 5 p.f.u./cell with HSV-1 (Glasgow strain 17) or HSV-2 (strain HG52) in Eagle's medium with 10% (v/v) calf serum and incubated at 37 °C for 8 h or 31 °C for 13 h. When necessary, H-dThd was added to infected cells for a variety of periods before harvesting both as an assessment of the efficiency of the infection and as a stability and density marker in subsequent operations. The cell sheets were washed, scraped from the growing surface and first nuclei and then chromatin prepared using the method of Rizzo & Bustin (1977). This technique involves hypotonic rupture of cells followed by washing of the nuclei with 1% (v/v) Triton X-100. After swelling in 20 mM-EDTA, nuclei were broken in a motor-driven Teflon homogenizer, crude chromatin centrifuged out, washed with 0.14 M-NaCl and pelleted through 1.7 M-sucrose containing 0.2% (v/v) Triton X-100. Before use, chromatin was washed three times and swollen in 5 mM-tris-Cl-, pH 7.9, 0.25 mM-EDTA.

Chromatin preparations were used at once, but could be stored for up to 24 h at 0 °C and for longer at −70 °C without substantial loss of activity. DNA synthesis was measured using the reaction mixture of Francke (1977a) and is as follows: 2 mM-ATP; 100 μmol each of CTP, GTP, UTP, dATP, dCTP, TTP; 20 μmol-dGTP; 1 mM-DTT; 1 mM-EGTA; 40 mM-HEPES (or tris-Cl−) pH 7.9; 5 mM-MgCl2; 80 mM-potassium acetate and α-32P-dGTP (> 350 Ci/mmol, 25 to 75 μCi/ml). dATP and TTP were also used as labels, on occasion. Incubation at 37 °C was terminated by the addition of 0.85 ml 20 mM-HEPES, pH 7.9, 10 mM-EDTA, 2% (w/v) SDS and 500 μg/ml boiled pronase and digested overnight at
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37 °C. Each sample was analysed by CsCl gradient centrifugation to separate virus and cell DNA using either the Beckman 50 Ti rotor at 35,000 rev/min for 65 h at 25 °C or the Sorval TV-865 rotor at 40,000 rev/min for 18 to 24 h at 25 °C. Gradients were fractionated by bottom puncture and samples precipitated on to paper discs and washed with acid and ethanol before scintillation or Cerenkov counting using standard techniques (Francke et al. 1978).

In the first series of experiments, nuclei and chromatin from uninfected and HSV-1 infected cells were assayed for DNA synthesis at a number of times of incubation in vitro. The results (Fig. 1) indicate that infected nuclei make both virus and cell DNA, while uninfected nuclei incorporate very poorly. Chromatin isolated from these nuclear preparations behaves in an analogous fashion, with, in the infected situation, both virus and cell DNA synthesis increasing with time. In this case the amount of chromatin used in the assay shown in Fig. 1 (c to f) was prepared from the amount of nuclei used for Fig. 1 (a, b). Thus preparation of chromatin from nuclei does not involve a substantial loss of synthetic ability, although in making the comparison we have not considered factors such as destruction of bound pools during manipulation. The impression from a number of such experiments is that the apparent rate of synthesis is falling by 30 min and has stopped by about 60 min, but incorporated label is stable (to acid precipitation) up to about 2 h. Similar results have been obtained with HSV-2 infected cells. Incorporation into cell DNA seems in many experiments to rise for longer than virus DNA, and this may reflect a differential stability similar to that described by Francke (1977b) in nuclei. At present we have only limited information on the properties of the virus DNA synthesized in chromatin preparations, but the maximum rate of synthesis (calculated at times of linear rise) is 20 to 30 pmol/min/10^7 nuclear equivalents, approaching that described by Bolden et al. (1975) and Francke (1977a) in isolated nuclei, and lower than that quoted by Shlomai et al. (1977) and Kolber (1975).

Investigation of DNA synthesis in vivo compared to in vitro incorporation in chromatin prepared at several times after infection shows a clear relationship, with cell DNA synthesis declining in vitro in parallel with the in vivo system (data not shown). Chromatin prepared from infected cells at late times of infection (later than about 15 h at 31 °C) synthesizes almost entirely virus DNA: we have tended to use chromatin prepared at earlier times, when both types of DNA are made.

Fig. 2. shows the effect of various treatments on the synthesis of DNA in chromatin from HSV-1 infected cells. Fig. 2 (h) suggests that the synthesis requires all four deoxyribonucleoside triphosphates, since lack of one (TTP) gives over 90% inhibition of both cell and virus DNA synthesis. Antiserum made against HSV-infected cell lysates (Hay et al. 1976) also has a strong inhibitory effect on both types of DNA synthesis, while pre-immune serum has little effect (Fig. 2 c, g): this indicates that HSV-specific antigen(s) are necessary for the synthesis of both cell and virus DNA in this system, and is supported by the effect of phosphonoacetic acid (PAA; Fig. 2 e), an inhibitor of the virus-induced DNA polymerase (Mao et al. 1975). This contrasts with the situation in vivo in which incubation of infected cells with PAA inhibits virus DNA synthesis but has little effect on the synthesis of cell DNA (Overby et al. 1974). Confirmation of the role of HSV DNA polymerase activity in the synthesis of both cell and virus DNA was obtained when chromatin was prepared from cells infected with a phosphonoacetate-resistant mutant (3) of HSV-1 (Hay & Subak-Sharpe, 1976). In these preparations both cell and virus DNA synthesis were resistant to the addition of a range of phosphonoacetate concentrations, in contrast to the wild-type situation. A typical result (at 10 μg/ml PAA) is shown in Fig. 2 (b, f). The nature of cell DNA synthesized by the virus DNA polymerase has not been investigated, but it is worth
Fig. 1. Time course of DNA synthesis in isolated nuclei and preparations of chromatin. BHK cells were infected with HSV-1 at m.o.i. of 5 p.f.u./cell for 8 h at 37°C. [3H-dThd (10 μCi/ml) was added to growth medium between 2 and 8 h after infection. Nuclei and chromatin were prepared as detailed in the text and incubated in the in vitro DNA synthesis assay as described in the text for a variety of times. The curves represent the results of CsCl gradient analysis of each sample: the denser fractions of each gradient are at the left. (a) Nuclei, mock-infected, 30 min at 37°C; (b) nuclei, infected, 30 min at 37°C; (c) chromatin, mock-infected, 30 min at 37°C; (d) chromatin, infected, 0 min at 37°C; (e) chromatin, infected, 10 min at 37°C; (f) chromatin, infected, 30 min at 37°C. [Total incorporation in (f) is 580 pmol.] ○—○, [3H incorporation (prior label)]; •—•, [32P incorporation (in vitro label)].
Fig. 2. Effect of various treatments on DNA synthesis in chromatin preparations. Chromatin was prepared from cells infected for 13 h at 31 °C with either HSV-1 wild type (wt) or a PAA-resistant mutant (31Pl) of HSV-1 and incubated in vitro as described in the legend to Fig. 1. The curves represent CsCl gradient analyses, with the denser fractions to the left. (a) Chromatin from HSV wt infected cells, 30 min at 37 °C. (b) Chromatin from HSV PAA-treated infected cells, 30 min at 37 °C. (c) Chromatin from HSV wt infected cells, 30 min at 37 °C, plus pre-immune serum. (d) Chromatin from HSV wt infected cells, 30 min at 37 °C, plus 10 mM-ATP (with the Mg²⁺ concentration raised to equimolar). (e) Chromatin from HSV wt infected cells, 30 min at 37 °C, plus 10 μg/ml phosphonoacetate. (f) Chromatin from HSV PAA-treated infected cells, 30 min at 37 °C, plus 10 μg/ml phosphonoacetate. (g) Chromatin from HSV wt infected cells, 30 min at 37 °C, plus anti-HSV antiserum. (h) Chromatin from HSV wt infected cells, 30 min at 37 °C, no TTP. Total incorporation in (a) represents 500 pmol.

noting once more that incorporation in vitro falls in parallel with incorporation in vivo. Finally, very high levels of ATP (Fig. 2d) also have a severe inhibitory effect, and the reasons for this are at present not clear. Removal of CTP, GTP and UTP from incubation mixtures inhibits DNA synthesis by about 50%, and omission of ATP alone has a slightly greater effect (data not shown).

Criteria of purity for chromatin preparations are difficult to establish, but we have subjected our preparations to light microscopy after staining and to electron microscopy after thin-sectioning and negative staining (uranyl acetate/lead citrate). Both techniques suggest the appearance of typical chromatin with no whole nuclei or visible amounts of nuclear membrane present: infected cell chromatin preparations contain numbers of full and empty virus capsid structures.

Thus, chromatin from infected nuclei seems to contain the bulk of the virus DNA synthetic ability of the infected cell: preliminary results of the size and replication characteristics of virus DNA are encouraging and suggest that such a system may be of value in the resolution of the mechanism of HSV DNA synthesis.
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