PREFERENTIAL ASSOCIATION OF URACIL–DNA GLYCOSYLASE ACTIVITY WITH REPLICATING SV40 MINICHROMOSOMES

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1. Introduction

Uracil–DNA glycosylase releases free uracil from dUMP-residues in DNA ([1–5], reviewed [6]). Uracil in DNA may arise from misincorporation of dUMP during DNA replication [7–9] or deamination of cytosine in DNA [10]. Much information is available on the biophysical and biochemical properties [1–6] of uracil–DNA glycosylase, but little is known about how this enzyme is organized in chromatin. Isolated SV40- or polyoma minichromosomes [11–13] serve as useful models for studies on chromatin proteins. Thus, DNA polymerases α and γ [13,14–17] as well as T-antigen [18,19] are associated with replicating SV40 and/or polyoma minichromosomes, while DNA polymerase β and topoisomerase I cosedimented with mature minichromosomes [17]. Here, we show that uracil–DNA glycosylase, a major DNA repair enzyme, is preferentially associated with replicating SV40 minichromosomes.

2. Materials and methods

Chemicals and reagents were obtained as in [5]. Growth of CV-1 cells and infections with SV40, strain Rh 911 (40 pfu/cell) were done as in [20]. The virus titre was measured in plaque assays [21].

A nuclear extract free of cellular DNA, but containing SV40 minichromosomes, was prepared from SV40-infected CV-1 cells at 36 h after infection when the rate of viral DNA synthesis was maximal [11]. The crude nuclear extract was centrifuged at 15 000 × *g* for 45 min at 4°C to remove mitochondria that contaminated the SV40 chromatin preparation.

To separate replicating and mature minichromosomes, 0.5 ml extract was layered on a linear 5–30% sucrose gradient in 10 mM Hepes (pH 7.8), 5 mM KCl and 0.5 mM dithiothreitol [11] and centrifuged at 37 000 rev./min in a Beckman SW 40 rotor for 150 min at 4°C. Fractions of 0.34 ml were collected from the bottom of the tube.

Uracil–DNA glycosylase was tested by incubating aliquots of 30 μl from the fractions with 10 μl assay buffer (200 mM NaCl, 8 mM EDTA, 160 mM Tris–HCl (pH 7.5) and 12 μM d[^{3}H]UMP-containing DNA, spec. act. 500 μCi/μmol) for 45 min at 30°C. The release of acid- or ethanol-soluble radioactivity was monitored [5]. The amount of radioactivity released did not exceed 30% of the added radioactivity. The release of [^{3}H]uracil was linear with time (up to 45 min) and with the amount of extract added (up to 30 μl). Cytochrome *c* oxidase [22] was determined in aliquots of 100 μl from extracts or gradient fractions. The detection limit was 0.025 nmol cytochrome *c* oxidized/min at 25°C.

3. Results and discussion

To label both replicating and mature SV40 chromatin, SV40-infected CV-1 cells were incubated with [^{14}C]thymidine (10 μCi/ml, spec. act. 56 mCi/mmol) for 20 min at 37°C. Nuclear extracts were then prepared, and replicating and mature minichromosomes separated by sedimentation in sucrose gradients (see section 2). To locate the chromatin peaks, aliquots of 100 μl from each fraction were acid-precipitated and the radioactivity measured by scintillation counting. Uracil–DNA glycosylase activity was measured in each fraction (section 2). Fig.1 shows that uracil–DNA glycosylase cosediments with replicating chromatin,

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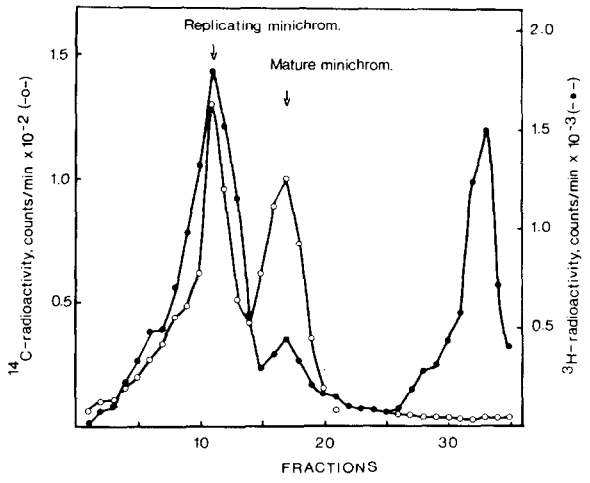

Fig.1. Cosedimentation of SV40 minichromosomes and uracil–DNA glycosylase activity. A nuclear extract was prepared from SV40-infected CV-1 cells labelled with [^{14}C]thymidine (10 μCi/ml, 56 mCi/mmol) for 20 min at 37°C prior to cell harvest. Replicating and mature minichromosomes were then separated by sedimentation in a sucrose gradient. Acid precipitable ^{14}C-radioactivity was determined [8] in aliquots of 100 μl from each fraction (–o–). Uracil–DNA glycosylase activity was determined in aliquots of 30 μl from each fraction (–•–).

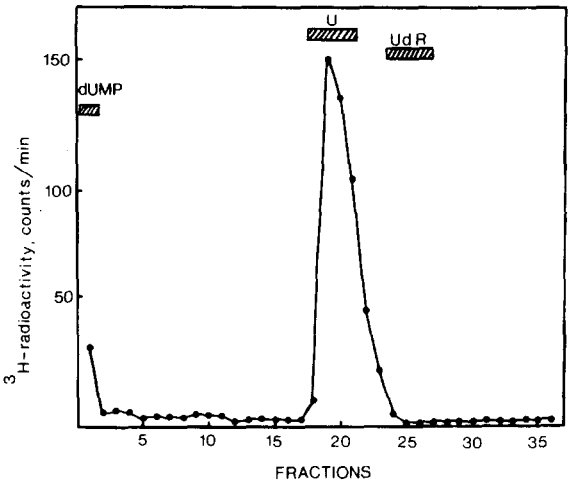

Fig.2. Thin-layer chromatography of ethanol-soluble radioactivity released. An aliquot of 30 μl from fraction 11 (fig.1) was incubated with 10 μl of uracil–DNA glycosylase assay mixture for 45 min at 30°C. Then cold ethanol was added to 70% (by vol.) and after 30 min at –20°C the tube was centrifuged (15 000 rev./min, 4°C, 5 min) and the supernatant collected. An aliquot of 8 μl was mixed with non-radioactive uracil, deoxyuridine and dUMP (20 nmol of each), applied to a sheet of polyethylene imine-cellulose and developed with H_2O. The lane was cut in 5 mm pieces and the radioactivity was extracted and counted as in [8].

although a smaller fraction cosediments with mature chromatin. About 34% of the total activity recovered was found close to the top of the gradient representing free uracil–DNA glycosylase. To establish the nature of the enzyme activity that made the radioactivity acid- and ethanol-soluble, an aliquot from fraction 11 was incubated with d[^{3}H]UMP–DNA and samples of the ethanol-soluble radioactivity released analyzed by thin-layer chromatography. About 90% of the radioactivity comigrated with uracil (fig.2). Furthermore, when d[^{3}H]TMP-containing, sonicated SV40 DNA was incubated with an aliquot from fraction 4, no radioactivity was made acid-soluble. No cytochrome *c* oxidase activity (<0.025 nmol cytochrome *c* oxidized/min by 100 μl of fraction 11) cosedimented with SV40 minichromosomes. Therefore, the cosedimentation of uracil–DNA glycosylase with replicating chromatin is not due to contamination by mitochondria, which are known to contain uracil–DNA glycosylase [23]. The most likely explanation for our observations is therefore that uracil–DNA glycosylase is preferentially associated with replicating SV40 minichromosomes.

The preferential, but not exclusive, association of uracil–DNA glycosylase with replicating chromatin may indicate that misincorporation of dUMP is a much more frequent event than cytosine deamination. A several-fold increase in uracil–DNA glycosylase activity in lymphocytes after mitogen stimulation may point to the same conclusion [24]. However, it is likely that cytosine deamination is potentially more harmful since it leads to a GC–AT transition mutation unless the lesion is repaired before the next round of DNA replication.

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References

[1] Lindahl, T., Ljungqvist, S., Siegert, W., Nyberg, B. and Sperens, B. (1977) J. Biol. Chem. 252, 3286–3294.
[2] Cone, R., Duncan, J., Hamilton, L. and Friedberg, E. C. (1977) Biochemistry 16, 3194–3201.

[3] Caradonna, S. J. and Cheng, Y.-C. (1980) J. Biol. Chem. 255, 2293–2300.
[4] Talpaert-Borlé, M., Clerici, L. and Campagnari, F. (1979) J. Biol. Chem. 254, 6387 6391.
[5] Krokan, H. and Wittwer, C. U. (1981) Nucleic Acids Res. 9, 2599–2613.
[6] Lindahl, T. (1979) Prog. Nucleic Acids Res. Mol. Biol. 22, 135–192.
[7] Tye, B.-K., Nyman, P. G., Lehman, I. R., Hochhauser, S. and Weiss, B. (1977) Proc. Natl. Acad. Sci. USA 74, 154–157.
[8] Wist, E., Unhjem, O. and Krokan, H. (1978) Biochim. Biophys. Acta 520, 253–270.
[9] Grafstrom, R., Tseng, B. and Goulian, M. (1978) Cell 15, 131–140.
[10] Lindahl, T. and Nyberg, B. (1974) Biochemistry 13, 3405–3410.
[11] Su, R. T. and DePamphilis, M. L. (1976) Proc. Natl. Acad. Sci. USA 73, 3466–3470.
[12] Su, R. T. and DePamphilis, M. L. (1978) J. Virol. 28, 53–65.
[13] Waldeck, W., Spaeren, U., Mastromei, G., Eliasson, R. and Reichard, P. (1979) J. Mol. Biol. 135, 675–689.
[14] Edenberg, H. J., Anderson, S. and DePamphilis, M. L. (1978) J. Biol. Chem. 253, 3273–3280.
[15] Otto, B. and Fanning, E. (1978) Nucleic Acids Res. 5, 1715–1728.
[16] Tsubota, Y., Waqar, M. A., Burke, J. F., Milawetz, B. I., Evans, M. J., Kowalski, D. and Huberman, J. A. (1978) Cold Spring Harbor Symp. Quant. Biol. 43, 693–704.
[17] Gourlie, B. B., Pigiet, V., Breaux, C. B., Krauss, M. R., King, C. R. and Benbow, R. M. (1981) J. Virol. 38, 826–832.
[18] Segawa, M., Sugano, S. and Yamaguchi, N. (1980) J. Virol. 35, 320–330.
[19] Reiser, J., Renart, J., Crawford, L. V. and Stark, G. R. (1980) J. Virol. 33, 78–87.
[20] Wilson, J. H., DePamphilis, M. L. and Berg, P. (1976) J. Virol. 20, 391–399.
[21] Mertz, J. E. and Berg, P. (1974) Virology 62, 112–124.
[22] DeDuve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. and Appelmans, F. (1955) Biochem. J. 60, 604–617.
[23] Anderson, C. T. M. and Friedberg, E. C. (1980) Nucleic Acids Res. 8, 875–888.
[24] Sirover, M. (1979) Cancer Res. 39, 2090–2095.