Estimation by Reassociation Assay of Viral DNA Copies in Three Polyoma Virus Transformed Cell Lines

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The number of polyomavirus genome equivalents in three transformed hamster cell lines were shown by DNA reassociation assay to be 1.3, 1.9 and 2.5 per cell.

We have determined the number of polyoma virus (PV) genome equivalents in PV transformed cell lines. The method used for estimation was the reassociation assay [1] of denatured ^{3}H-labelled DNA from transformed cell lines.

^{3}H-labelled PV DNA was obtained by labelling of PV infected (polyoma wild type virus, a gift from Dr. J. Žemla, Bratislava) secondary mouse embryo cells with [6-^{3}H]thymidine [50 μCi ml^{-1} in the presence of fluorodesoxyuridine (10 μg ml^{-1})] and DNA isolation according to Hirt [2]. Purification of PV DNA was achieved by ribonuclease treatment, a two-fold ethanol precipitation, and three centrifugation steps (CsCl sedimentation, alkaline sucrose sedimentation, and CsCl ethidium bromide equilibrium centrifugation) [3]. We used form I of PV DNA. The DNA was sheared by sonication (4 to 6S) and denatured by heating for 10 min at 100 °C and rapid cooling.

Cell DNA was prepared according to Colter *et al.* [4] from three PV transformed cell lines. The lines PyBHK and Pyts3-BHK were obtained from Dr. H. Werchau (University Bochum), and line PyBHK R.C. from Dr. H. Türler (Geneva).

After shearing and denaturation, the DNA was reassociated with ^{3}H-labelled PV DNA at 66 °C in one-ml probes containing sodium phosphate (pH 6.9) and 0.4% SDS.

After different incubation periods, aliquots were drawn from the incubation mixtures and diluted with H_2O to obtain a phosphate concentration of 0.14 M. DNA reassociation was monitored by DNA fractionation on hydroxyapatite columns (2 ml volume; DNA grade Biogel HTP from BioRad Laboratories). Single-stranded DNA was obtained in the 0.14 M phosphate (0.4% SDS) column eluates and double-stranded DNA in the 0.4 M phosphate eluates. After DNA precipitation with TCA on glass fiber filters, radioactivity was measured by scintillation counting.

The reassociation assay was first calibrated with ^{3}H-labelled PV DNA (in the presence of 2 μg ml^{-1} salmon sperm DNA) and then the increase of ^{3}H-PV DNA reassociation after addition of DNA from the transformed cell lines was determined. The reassociation data obtained are plotted in a graph (Fig. 1).

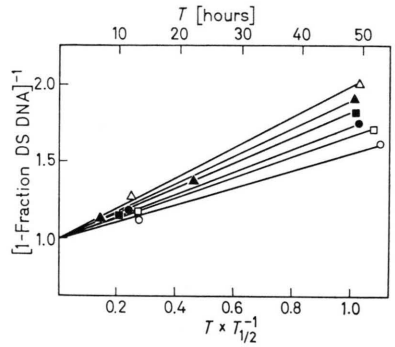

Fig. 1. Reassociation kinetics of PV DNA. Open symbols: Reassociation of PV DNA in the presence of 1.75 mg salmon sperm DNA; (○), 3.25 ng ^{3}H-PV DNA (spec. radioact. 1.2×10^3 cpm ng^{-1}); (□), 3.25 ng ^{3}H-PV DNA plus 0.65 ng of unlabelled PV DNA; (△), 3.25 ng ^{3}H-PV DNA plus 1.95 ng of unlabelled PV DNA. Filled symbols: Reassociation of 3.25 ng ^{3}H-PV DNA in the presence of 1.75 mg cell DNA from line (●), PyBHK; (■), Py ts3 BHK; (▲), Py BHK R.C.

Table I. Number of PV DNA copies per transformed cell DNA equivalent.

Cell line	Increase in the slope * of the reassociation curve per 1.75 mg of cell DNA	PV DNA equivalent per one cell DNA equivalent
PyBHK	0.18	1.3
Py-ts3 BHK	0.26	1.9
Py BHK R.C.	0.34	2.5

* Slope per 3.25 ng PV DNA is 0.58 (mean).

The calculation (Table I) of PV genome content of the transformed lines is based on the slope of the reassociation curve (Fig. 1) and on the molecular weights of cell DNA (4.0×10^{12} dalton for the diploid genome) and of PV DNA (3.0×10^6 dalton) [5, 6]. The PV genome content of the three investigated transformed lines was in the range of 1.3 to 2.5 PV equivalents per one cell genome.

This estimation is in conformity with recently published results of Kamen *et al.* [7], who found 2.0 and 2.4 PV genome equivalents in the lines 4198 (C3H mouse) and PY 6 (3T3 mouse), respectively.

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Similar data have been obtained in reassociation assay for the majority of SV40 transformed lines [6].

Earlier investigations with PV transformed lines by means of filter hydridization of *in vitro* transcribed labelled complementary RNA with unlabelled DNA from the transformed cell lines indicated a markedly higher number of PV DNA copies (6 to 9) per transformed host cell DNA [5, 8].

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