ASSOCIATION OF SV40 DNA SEQUENCES WITH NUCLEAR MATRIX IN SV40 TRANSFORMED HAMSTER FIBROBLASTS

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Received 30 November, 1986

Nuclear matrices were isolated by the high-salt, non-ionic detergent method from SV40-transformed hamster fibroblasts (TSV5 cell line), and from hamster tumours derived from these cells. DNA isolated from matrices and total nuclei was hybridized with nick-translated SV40 DNA. The enrichment of matrix DNA with SV40 DNA sequences was observed in all five experiments with matrix DNA of TSV5 cells but only in five out of nine matrix DNA isolated from tumour cells.

Eucaryotic DNA is organized in topologically constrained domains or loops. These loops attached to the proteinaceous structure called nuclear matrix, might represent functional units of DNA restricted by the points of attachment. The average length of the loops correlates well with the length of replication and transcription units (see review by Zehnbauer & Vogelstein [1]). Spatial organization of DNA seems to be independent of chromatin proteins (e.g. histones) [2]. The nuclear matrix is defined as the residual structure obtained after extraction of the purified nuclei with non-ionic detergent, nucleases and high salt solutions. This structure is composed of the pore complex-lamina layer, the residual nucleoli, and the intranuclear network [3]. The nuclear matrix was reported to be enriched with newly synthesized DNA [4] and is thought to be involved in transcriptional activity [5, 6].

The large T antigen of SV40 virus is continuously expressed in hamster fibroblasts transformed with simian virus 40 (TSV5 cell line). We have studied the topological relations of SV40 DNA sequences to nuclear matrix in this cell line and in hamster tumours derived from TSV5 cells.
MATERIALS AND METHODS

Cells and tumours. TSV5 cells kindly supplied by Dr. R. Weil (University of Geneva) were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Tumours were obtained 8 weeks after subcutaneous injection of $1 \times 10^8$ TSV5 cells (14-18th passage) into four week-old Syrian hamsters.

Isolation of nuclear matrix. Matrix was isolated according to Berezney & Coffey [2], as modified by Krzyżowska-Gruca et al. [7]. In brief, tumours (3-7 g wet weight) were minced with scissors, homogenized in 0.25 M saccharose in buffer A (10 mM morpholine, pH 6.0, 10 mM NaCl, 1.5 mM MgCl$_2$), filtered through gauze and pelleted. TSV5 cells grown in plastic tissue culture bottles were scraped off and washed into buffer A. Both types of cells were lysed in 0.1% Triton X-100 and 0.2% sodium deoxycholate, homogenized with Dounce homogenizer tight A and pelleted. Nuclei were washed with TM3 buffer (50 mM Tris/HCl, pH 7.4, 3 mM MgCl$_2$) containing 0.25 M saccharose, incubated in TM3 containing 1% Triton X-100, washed with TM3 buffer, incubated with 1 mM CuCl$_2$ and washed 3 times with TM3 buffer. Nuclei stabilized with Cu$^{2+}$ were suspended ($2 \times 10^8$ nuclei/ml) in TM3 buffer containing 0.5 NaCl, then 100 µg/ml of DNAse I (Worthington) or 0.5 - 6 µg/ml of micrococcal nuclease was added and the mixture was incubated for 15 - 30 min at 37°C. Nuclear matrices were pelleted and washed successively with 1 M and 2 M NaCl in TM3 buffer.

All buffers contained 1 mM phenylmethylsulfonyl fluoride (PMSF). The preparation procedure was carried out at 0°C unless otherwise stated.

Isolation of nuclear matrix DNA. Nuclei and nuclear matrix preparations were incubated with proteinase K (Sigma) at a concentration of 50 µg/ml, for 1 h at 68°C, and DNA was extracted with phenol according to Maniatis et al. [8], digested with RNAase (Sigma), extracted with phenol, and precipitated with ethanol.

Isolation and labelling of SV40 DNA. For SV40 isolation, CV1 cells were infected with SV40 virus (0.01 pfu per cell). Viral DNA was isolated according to Hirt [9], and form I of DNA was purified in CsCl (Merck) gradient according to Maniatis et al. [8]. Purified SV40 DNA was dialysed twice against 50 mM Tris/HCl, pH 7.4, containing 3 mM MgCl$_2$, precipitated with ethanol and used for nick translation. SV40 DNA was labelled with $\alpha$-$^{32}$P dATP (Amersham, 7000 Ci/mmol) to a specific activity of $1 - 5 \times 10^7$ cpm/µg by nick-translation according to Rigby et al. [10]. The nonincorporated nucleotides were removed from the reaction mixture by passage through Sephadex G-50 (Pharmacia) column, and DNA was recovered by ethanol precipitation.
Hybridization of SV40 DNA to matrix and nuclear DNA. Total and nuclear matrix DNA denatured at 100°C was mixed with the same volume of 20 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M Na citrate) and 20 µl samples were dot-blotted with a 50 µl capillary onto GeneScreen membranes (New England Nuclear). The retention of DNA on GeneScreen membrane was from 70 to 85%. Pre-hybridization was carried out at 60°C in 4 × SSC, 0.1% SDS, 5 × Denhardt solution [11] and 100 µg/ml of salmon sperm DNA. Denatured, nick-translated SV40 DNA (100 µl, 10⁷ cpm) was added to the pre-hybridization solution, and hybridization continued for 18 - 20 h. Filters were then washed successively with 7 M urea, 2 × SSC, 0.2% SDS at 42°C and with 2 × SSC, 0.2% SDS at 60°C. After drying, the filters were exposed for 2 - 14 days to Kodak X-AR 5 films at −70°C. All scannings were performed with CDS-200 Computing Densitometer System (Beckman) within the linear portion of the pre-established calibration curve.

Determination of proteins and DNA. Protein content in different fractions was determined by the standard method of Lowry et al. [12] and DNA was assayed spectrophotometrically.

RESULTS AND DISCUSSION

Nuclear matrices contained 8 -12% of the proteins and 2 -15% of the DNA present in the intact nuclei: the range depended on the amount of the nucleolytic enzyme used and time of incubation.

The matrix DNA from TSV5 cells contained 3 - 16 times more SV40 DNA sequences than total nuclear DNA (Table 1, Fig. 1). There is a negative correlation between enrichment indices and the percentage of DNA recovered with the matrix: the highest (16%) enrichment with SV40 DNA sequences

<table>
<thead>
<tr>
<th>Experiment No</th>
<th>% of DNA recovered in matrix</th>
<th>Enrichment* with SV40 DNA sequences</th>
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<tr>
<td>1</td>
<td>0.5</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
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* Ratio of SV40 DNA hybridized with matrix DNA to SV40 DNA hybridized with total nuclear DNA.
was observed in the matrix preparation with the lowest matrix DNA content (0.5%). This observation is consistent with the concept that active genes are closely associated with nuclear matrix, and also with the fact that TSV5 cells in tissue culture are actively synthesizing large T antigen. Our results corroborate the results of Nelkin et al. [13], who observed enrichment of nuclear matrix from SV40-infected 3T3 cells with SV40 DNA. Transcriptionally active SV40 DNA sequences were associated with nuclear matrix [14] and some nuclear matrix proteins were copurified with SV40 chromosomes [15]. In an early phase of infection of HeLa cells with adenovirus type 5, nuclear matrix was enriched several fold with the adenoviral DNA [16].

Several cell lines transformed by other viruses, e.g. polyoma or avian sarcoma viruses contain integrated viral genes attached to the nuclear matrix [7]. Moreover, this attachment closely correlates with expression of viral genes and transformed phenotype. Therefore the results obtained with the tumours derived from TSV5 cells implanted into hamsters were rather unexpected. In only five tumours out of nine the matrix DNA was enriched 2.3-6 times with SV40 DNA sequences (Table 2, Fig. 2) and, similarly to TSV5 cells grown in culture, matrices containing the lowest percentage of DNA showed the highest enrichment index. In four
tumours nuclear matrices were practically depleted or SV40 DNA. (Table 2, Fig. 2). The possibility of experimental error connected with the fact that the retention of DNA on GeneScreen membrane was never complete should be taken into account. Also artefacts introduced in matrix preparation by high salt concentration [18, 19, 20] may obscure the picture. However, in the light of experiments with TSV5 cells where in the same conditions we observed repeatedly the enrichment of matrix DNA with SV40 sequences, it seems that the depletion phenomenon resulted rather from individual

Table 2

*Enrichment with SV40 DNA in matrix preparations isolated from hamster tumours*

<table>
<thead>
<tr>
<th>Experiment No</th>
<th>% of DNA recovered in matrix</th>
<th>Enrichment** with SV40 sequences</th>
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** See footnote to Table 1.

Fig. 2. Densitometry scanning of DNA dot-blot assay of nuclear and matrix DNA from hamster tumour cells: A) tumour nuclear matrix DNA preparations enriched 6 times with SV40 DNA, B) one of four tumour matrix DNAs where no enrichment with SV40 DNA is seen. Each dot has 3 μg of either total or matrix-associated DNA.
differences among tumour tissues (cell heterogeneity, tumor progression) than from the procedural error. One can assume that DNA attachment to matrix may change during cell cycle and depends upon the physiological state of the cell [16, 21, 22]. In this case depletion of matrix of SV40 DNA during tumour growth cannot be excluded. Moreover, there is evidence that in the course of tumour progression deep rearrangements of DNA sequences take place that can change topology of DNA. For example, the rearrangement of SV40 DNA was observed in the consecutive passages of Balb/c-3T3 cells [23].

Significance of the nuclear matrix in the function of genome is not fully understood. Although numerous data indicate that the actively transcribed genes are closely associated with nuclear matrix, as it was found for genes encoding globin in duck erythroblasts [24], ovalbumin in chicken oviduct [25], histones and heat shock protein 70 (hsp70) in Drosophila cells [19], it is not unequivocally excluded that genes can be transcribed without being associated in nuclear matrix. In 1985 Small et al. [26] found that the transcription rate of hsp70 genes in Drosophila cells was not related to their association with in the matrix.

Also it is known that the role of nuclear matrix is complex. The matrix serves as an attachment point for estrogen receptors [27], some onc proteins [28], DNA polymerases and topoisomerase I [29], RNA polymerase II [30] and DNA methylase [31]. The major protein component of the Drosophila matrix has been identified as topoisomerase II [32, 33]. It seems that much more information is needed to explain the function of this enigmatic structure and its role in the cell.

REFERENCES


