THE SV40-INDUCED T-ANTIGEN IN MOUSE CELLS*

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1. Introduction

The size of the SV40 genome (3.2×10^6 daltons) [1] is too small to code for the many new virus-specified proteins that are observed after SV40 infections and transformation.

Transformation of cells by SV40 is correlated with the integration of one or more viral genomes into the cellular DNA [2]. The transformed cells do not produce infectious virus, i.e. they do not synthesize the viral structural proteins, but they exhibit a number of viral functions or virus-induced host functions such as T- (tumour), s- (surface), TSTA- (tumour-specific transplantation antigen) antigens, Phytoagglutinin-binding sites and the foetal or carcinoembryonic antigens.

Concerning the T-antigen, it is known whether this antigen is a virus- or host-coded antigen. Since the T-antigen is found in SV40-transformed cells of different animal species, it was assumed to be virus-coded. But it has not been ruled out that it is a derepressed cross-species-reacting protein found in cells from various species. In this case it would be coded for by an adult derepressed host gene.

As a second possibility it could be a product of a foetal, derepressed host gene. This second possibility was shown to be true for thymidine kinase by Bull et al. [3]. They show that transformation with SV40 might be associated with derepression of foetal gene expression for thymidine kinase. Furthermore, a third mechanism, namely the modification or alteration of cellular proteins has to be considered. The third explanation can be combined with either one of the two first mechanisms.

The experiments described in this paper were initiated to extend the knowledge about the nature and the origin of the T-antigen.

2. Materials and methods

2.1. *Cell culture conditions*

3T3 and SV3T3 cells were maintained in Eagle's basal medium with twice the concentration of amino acids and vitamins (10% foetal bovine serum for 3T3, 5% for Sv3T3). According to three different techniques the cells were free of *Mycoplasma:* electronmicroscopy, [^{3}H]thymidine uptake shown in autoradiography and a biological test (Mycoplasma test, Flow Laboratories). For isotope incorporation ^{14}C-labeled amino acids (*Chlorella* protein hydrolysate, specific activity 45 mCi/mmole atom of carbon) or a mixture of ^{3}H-labeled amino acids which consisted of equal activities of the following amino acids: L-[4,5-^{3}H]-lysine, specific activity 250 mCi/mmole; L-[4,5-^{3}H] leucine, specific activity 1.000 mCi/mmole; L-[2,3-^{3}H] valine, specific activity 1.500 mCi/mmole, (The Radiochemical Centre, Amersham, England) was added.

Buffers: 0.001 M NaCl; 0.0015 M $MgCl_2$; 0.01 M Tris–HCl, pH 7.4 (reticulocyte standard buffer = RSB); 0.12 M NaCl; 0.018 M Na_2HPO_4; 0.0025 M KH_2PO_4, pH 7.2 (phosphate-buffered saline = PBS).

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2.2. *Protein analysis and preparation of sera*

Protein preparation, polyacrylamide gel electrophoresis, and assay for radioactivity was done as described previously [4]. Molecular weight determination was done according to Shapiro [5] with the following enzymes of known molecular weight: lysozyme, phosphorylase *a*, L-amino acid oxidase, D-amino acid oxidase, trypsin.

Sera against T-antigen were obtained from hamsters bearing SV40-induced tumours. Control sera were taken from normal hamsters. All sera were adsorbed against rabbit liver–kidney powder.

3. Results and discussion

Experiments were done with uncloned, cloned and newly transformed cells. With respect to the growth characteristics (fig. 1) cells were harvested according to the number of cells (N) and according to the time after seeding (T). This was done in order to compare them with the pulse-labeling experiments. The specific activity was calculated prior to loading the gels. No significant difference was found between cells harvested according to the number or cells harvested according to time (fig. 1).

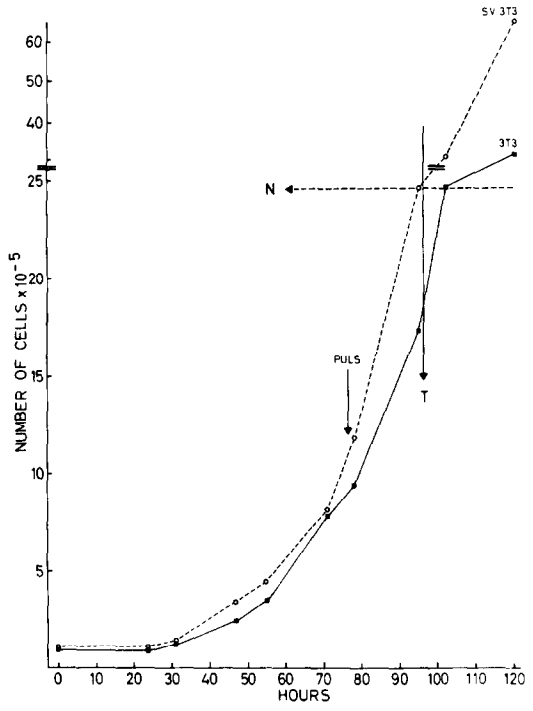

Fig. 1. Growth of normal mouse cells (3T3) and SV40 transformed mouse cells (SV3T3). 2×10^5 cells were seeded and counted at intervals indicated. For pulse-labeling experiments cells were harvested with respect to the number of cells (N) or the time after seeding.

In a first series of experiments ^{3}H-labeled amino acid labeled (6 μCi/ml 3T3 cells were compared with ^{14}C-labeled amino acid labeled (0.6 μCi/ml) SV3T3 cells. Total cell proteins were prepared in 1% SDS according to Summers [6] and coelectrophoresed in a 7.5% gel (fig. 2). No significant alterations were observed in the high and medium molecular weight area. Only some quantitative differences in the low molecular weight area are found as shown by the shadowed areas in fig. 2.

Since we had a typical iceberg situation in this case, we started to look for specific proteins contained in the total protein preparation. The idea was to precipitate the expected T-antigen in the protein preparation of transformed SV3T3 cells.

The sera showed a brilliant, typical T-staining at a 1:10 dilution. This immunofluorescence was 100% positive for the SV3T3 and negative for 3T3.

The protein preparation described was made as follows: cells were harvested in RSB buffer, sonified with a Branson sonifier (step 4, 2 X 20 sec), centrifuged in a Spinco for 100 000 *g* (SW 27, 36 000 rpm, 10 min) at 4°C. Assuming that the T-antigen is in the supernatant, a double antibody technique was used. The supernatant from SV3T3 cells was incubated first with anti-T serum and subsequently with rabbit anti-hamster serum.

After incubation of the 100 000 *g* supernatant with the antisera, centrifugation was done at 3000 *g* for 30 min (4°C). The resulting pellet was washed twice with PBS buffer. At each purification step radioactivity was measured.

The final double antibody ^{14}C-labeled antigen-complex was disintegrated with SDS according to the standard procedure and run on a 7.5% gel. Two peaks were found with molecular weights of about 70 000 and 30 000 (fig. 3, upper panel). The radioactivity found was much less than 1% of the total cell radioactivity. The peaks are defined by the specificity of the anti-T serum mentioned above. The 70 000 mol. peak (NCVPO, table 1 and fig. 3) is consistent

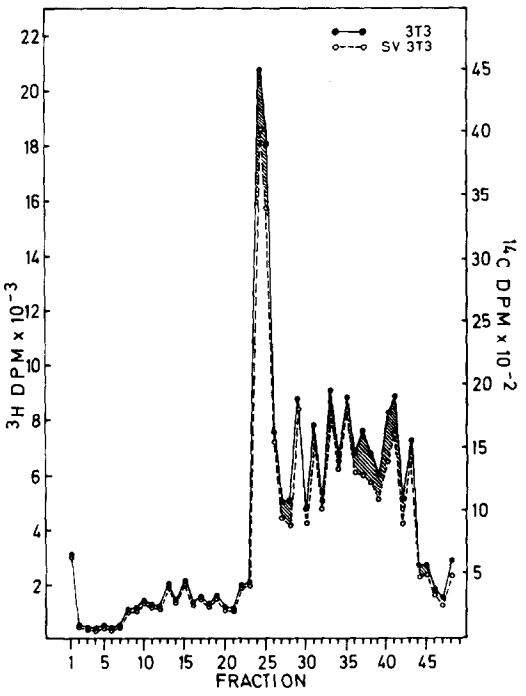

Fig. 2. Coelectrophoresis of the total cell protein preparation of ^{3}H-labeled 3T3 and ^{14}C-labeled SV3T3 cells (for details see text). The gels were loaded with 8 times as much ^{3}H counts as ^{14}C counts.

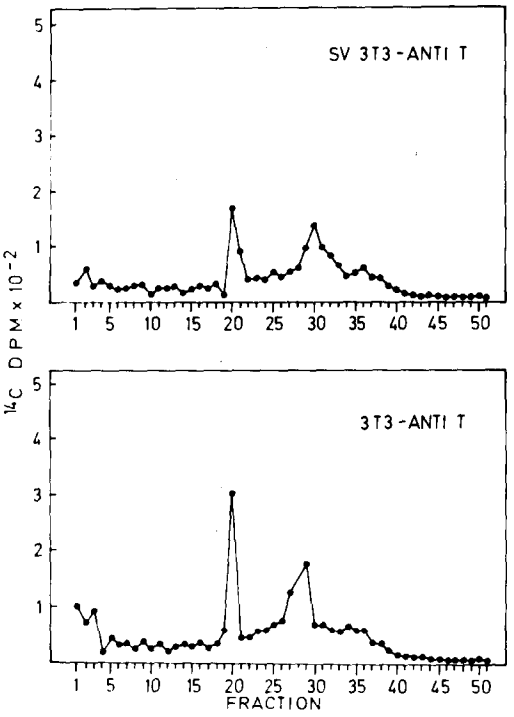

Fig. 3. SDS-polyacrylamide gel pattern of proteins precipitated with anti-T serum from 100 000 *g* supernatant of the total preparation from SV3T3 (upper panel) and 3T3 cells (lower panel). Cells were harvested in RSB buffer (for details see text).

with results by Walter et al. [7] and the peak with the low molecular weight is in accordance with NCVP1, an early protein which has been described by us for the lytic system [4]. From these findings we conclude that the peaks found are subunits of the SV40 T-antigen.

We performed the same experiment with normal 3T3 cells that were precipitated with anti-T (fig. 3, lower panel). No significant difference was observed compared to the transformed lines. This experiment was often repeated, but with the same surprising result. No precipitate was obtained with protein preparations from SV3T3 and 3T3 cells with sera from normal hamsters.

One interpretation of this finding might be that a pre-existing cellular T-antigen like protein, coded for by the host genome, exists in normal 3T3 cells. Again, we have to face the fact that a pre-existing T-antigen cannot be detected by indirect immunofluorescence due to the relative insensitivity of the assay This cellu-

Table 1
Molecular weights of SV40 polypeptides

Designation	Molecular weight	Lytic		Transformed SV3T3
		early	late	
NCVPO	70 000	+	–	+
VP 1	42 000	–	+	–
VP 2	32 000	–	+	–
NCVP 1	30 000	+	–	+
VP 3	23 000	–	+	–
NCVP 2	21 000	+	–	unknown
VP 4	14 000	–	+	–
VP 5	12 500	–	+	–

Markers with known molecular weight were used to estimate the molecular weights of the SV40 polypeptides on 7.5% SDS-polyacrylamide gels. VP = structural proteins of the virion, NCVP = proteins which are not part of the virion.

lar protein undergoes changes in its subunit structure as a result of the altered metabolic state. In table 1 the molecular weights of the known SV40 induced proteins are listed. It is interesting to note that NCVPO and NCVP1 occur in the lytic and in the transformed system as well (table 1).

According to Osborn and Weber [8] the size of T-antigen varies, depending on what mutants and temperature are used. Further experiments will give us a hint as to how T-antigen is built up out of subunits and whether or not the host component is specific.

I would like to speculate that the T-antigen consists of different subunits of cellular origin which are combined with an analogous viral subunit, or alternatively, the cellular subunits might be slightly modified by the virus.

Acknowledgements

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