HOMOLOGY BETWEEN SV40 DNA AND DNA OF NORMAL AND SV40-TRANSFORMED CHINESE HAMSTER CELLS

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SUMMARY

The extent of homology between simian virus 40 (SV40) DNA and DNA of normal and SV40-transformed Chinese hamster (ChH) DNA has been studied by DNA-RNA and by DNA-DNA hybridization. Evidence was found for the existence of some complementary sequence between SV40 DNA and ChH and CV-1 cell DNA. However, these complexes had a different thermal stability from those of SV40 DNA or SV40-complementary RNA (cRNA) with SV40-transformed cell DNA.

By the nucleic acid hybridization technique, it has been shown that in the case of some polyoma- and simian virus 40 (SV40)-transformed cells (1,2), the viral genome, or part of it, is covalently linked to the cell DNA. It is difficult to determine how much of the viral genome is present in the transformed cell since some degree of homology has been reported between SV40 DNA and the DNA of some normal cells (3,4). In the present studies, the extent of the homology between SV40 DNA and the DNA of normal and SV40-transformed Chinese hamster (ChH) cell DNA has been studied by DNA-RNA hybridization techniques, using SV40 complementary RNA (cRNA) synthesized *in vitro* by *E. coli* DNA-dependent RNA polymerase, and by DNA-DNA hybridization.

MATERIALS AND METHODS

The cells used were CV-1 cells (a monkey permanent line), primary Chinese hamster (ChH) embryo cells, and cells from two clones of SV40-transformed Chinese hamster cells, C1-5 and C1-21 (5). The technique for isolation of metaphase chromosomes was the one described by Maio and Schildkraut (6). Strain Rh 911 of SV40 grown in CV-1 cells was used.

The basic procedures used for purification of SV40 and SV40 DNA were those described by Carp et al. (7). After two KBr gradient centrifugations, the fractions containing the virus were dialyzed against 0.05 M tris (hydroxy methyl) amino methane (Tris buffer), pH 7.4, and subjected to equilibrium centrifugation in a CsCl density gradient (ρ = 1.34) at 36,000 rev/min (Beckman Spinco SW 39 rotor) for 20 hrs. The virus band was collected and dialyzed against the Tris buffer. SV40 DNA was extracted from this virus fraction by the trichloroacetic acid (TCA)-phenol method and chromatographed through a methylated albumin column (8).

Labeled SV40 DNA was prepared by adding 0.2 μCi per ml of tritiated thymidine (specific activity, 6 Ci/mole) to SV40-infected CV-1 cells and selectively extracted from the infected cells by the method of Hirt (9). The extracts were added to CsCl (1.566 g/ml) in a solution of 0.01 M Tris, pH 8.0, 0.01 M ethylenediaminetetraacetate (EDTA), and 100 μg/ml ethidium bromide, and centrifuged (Beckman Spinco 50 angle-head rotor) at 43,000 rev/min for 48 hrs at 20 C.

Cellular DNA was extracted from confluent monolayers of ChH and of CV-1 cells. The monolayers were washed by phosphate-buffered saline (PBS), pH 7.2, and lysed in a solution containing 1% sodium dodecyl sulfate (SDS), 0.1 M NaCl, and 0.1 M Tris-Cl buffer, pH 9.0 (10). The DNA was extracted from the lysate by two successive phenol treatments and precipitated from the aqueous phase with two volumes of cold ethanol. The thread-like precipitate was collected on a glass rod or by centrifugation and dissolved in 1X SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate). Cell DNA was then

purified by isopycnic centrifugation in CsCl (density 1.70 g/cm^3). DNA was denatured by bringing the solution to pH 12.8 with 10 N NaOH for 10 min and then neutralizing it to pH 7.0.

For the in vitro synthesis of SV40 complementary RNA (cRNA) the following mixture was used in 250 µl: 3 µg of DNA-dependent RNA polymerase prepared by the method of Chamberlin and Berg (11), 0.5 - 1.0 µg of SV40 DNA component I, 0.0016 M each of adenosine 5'-triphosphate (ATP), guanosine 5'-triphosphate (GTP), uridine 5'-triphosphate (UTP), 5-10 µCi of tritiated cytidine 5'-triphosphate (^{3}H-CTP) (13 Ci/mmole, Schwarz Bio-Research), 0.2 M KCl, 0.012 M $MgCl_2$, 0.012 M Spermidine, 0.05 M β-mercaptoethanol, 0.1 M Tris-acetate, pH 7.8.

After three hours of incubation at 37 C, the reaction was stopped by the addition of 4 µg of DNase for 30 min at 37 C, and then SDS at a 0.5% final concentration. The mixture was treated with phenol for 15 min at room temperature. The resulting emulsion was separated into two layers by centrifugation at 10,000 rev/min for 10 min and the upper aqueous phase containing the cRNA was dialyzed against SSC and stored at -25 C.

For DNA-RNA hybridization, the alkaline-denatured DNA on the membrane filter was incubated for 20 hrs at 65 C in a vial containing a 1-ml solution of 6X SSC, tritiated cRNA (1-2 X 10^5 cpm), and 0.1% SDS. During hybridization, the cellular DNA (10-150 µg) immobilized on the membrane was spontaneously released to the extent of 0-10%. There was no evidence of release of ^{3}H-SV40 DNA (0.001-0.1 µg per membrane) even after an incubation for 48 hrs at 65 C.

For DNA-DNA hybridization, alkaline-denatured DNA, immobilized on membrane filter, was incubated for 6 hrs at 65 C in a vial with 1 ml of a preincubation medium (3X SSC), containing 0.2% each of Ficoll (avg. mol. wt., 40,000 daltons), polyvinylpyrrolidone (avg. mol. wt., 360,000 daltons), and crystallized bovine albumin (12).

^{3}H-cellular or ^{3}H-SV40 DNA was fragmented by sonic treatment in SSC using a sonifier cell disruptor (Head Systems Co., Melville, L.I., N.Y.).

The DNA was then denatured by alkali, and the solution adjusted to 6X SSC. The labeled DNA solution was added directly to the vials containing filters with immobilized unlabeled DNA and preincubated medium, and incubated at 65 C for 18 hrs. After hybridization, the membrane filters were washed repeatedly in 2X SSC, dried, and counted in a Beckman liquid scintillation system.

RESULTS

Properties of ^{3}H-cRNA synthesized in vitro by E. coli DNA-dependent polymerase. The size of the ^{3}H-SV40 cRNA synthesized in vitro by E. coli DNA-dependent RNA polymerase on the SV40 DNA component I as template was determined by velocity sedimentation in sucrose gradient in 5-20% sucrose, 0.01 M Tris 0.001 M EDTA, 0.1 M NaCl, pH 7.4,after the ^{3}H-SV40 cRNA had been heated for 3 min at 65 C in the same solution and rapidly chilled. (Under these conditions, aggregation of the RNA is minimized.) (13). The size was rather heterogenous, with a peak of 10S and a broad distribution above and below this value.

The amount of SV40-cRNA bound is dependent on the concentration of immobilized SV40 DNA (Fig. 1). When SV40-cRNA was hybridized with CV-1 or normal ChH cell DNA, the amount of bound ^{3}H-SV40 cRNA was about the same as that for the blank filter. C1-21 and C1-5 cell DNA bound cRNA at levels higher than the background, particularly in the case of C1-21. Neither cell line contained any detectable infectious SV40.

In addition, the C1-21 DNA hybridizable with ^{3}H-SV40 cRNA had alkaline stable linkage to cellular DNA, as demonstrated by hybridization experiments after alkaline sucrose gradient (unpublished). Therefore, it appears that in C1-21 transformed cells, SV40 DNA is integrated into the cellular DNA. The same level of hybridization of ^{3}H-SV40 cRNA was observed when the DNA on the filter was obtained from the whole C1-21 cell or from C1-21 isolated chromosomes (Table 1), indicating that all of the

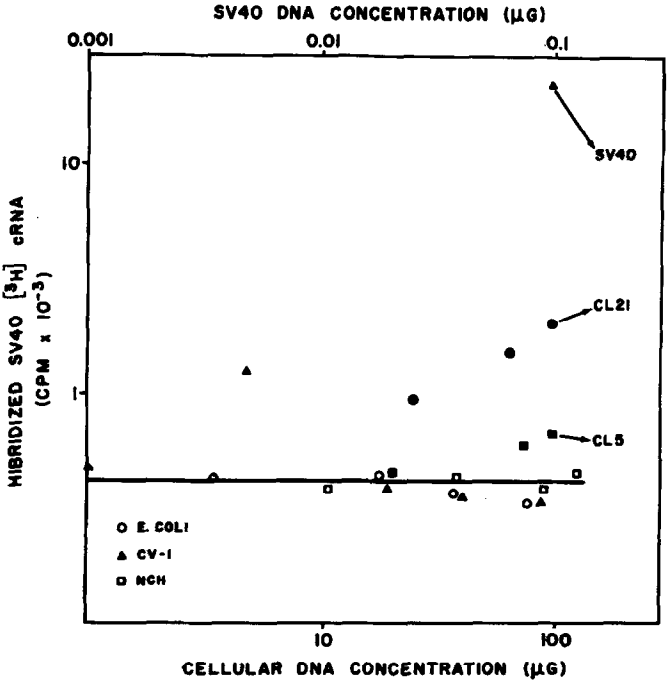

Fig. 1. SV40 cRNA hybridization with SV40 DNA and cellular DNA. The assay of DNA-RNA hybridization was carried out as described in Materials and Methods. The input radioactivity of ^{3}H-SV40 cRNA was 1.27 X 10^{5} cpm. The background (no DNA) was 411 cpm. This value has not been subtracted from the recorded data. The upper horizontal axis shows SV40 DNA concentration, while the bottom one shows DNA concentration of C1-21, C1-5, E. coli, CV-1 and normal ChH.

^{3}H-SV40 cRNA hybridizable DNA in these cells is associated to the chromosomes.

Heat stability of DNA-RNA complexes. Previous experiments indicate that some homology exists between ^{3}H-SV40 cRNA and cellular DNA of transformed cells. However, they do not give us any information about the size of the complementary nucleotide sequence. To solve this problem, the heat stability of the DNA-RNA complexes was investigated. The DNA-RNA complexes on the nitrocellulose membrane filters were treated with 10 μg/ml of pancreatic RNase A (Worthington Biochemical Co.) in 2X SSC, washed and incubated in 1 ml of 2X SSC for 10 min at the indicated temperature. The eluate from the filter was counted. During the thermal elution, only about 5-10% of the DNA was released from the filter. The heat stability

Table 1

Hybridization of SV40 cRNA with DNA extracted from cell and chromosome

DNA on the filter	cpm per 100 μg of DNA*
Normal ChH cell	95
C1-21 cell**	1333
C1-21 chromosome	1325

*Input ^{3}H-SV40 cRNA (1.27 X 10^{5} cpm).

**SV40-transformed ChH cells.

of DNA-RNA hybrids was essentially the same for [SV40-DNA + ^{3}H-SV40-cRNA] and [C1-21-DNA + ^{3}H-SV40-cRNA] hybrids, when the percentage of eluted radioactivity from the [c1-21-DNA + ^{3}H-SV40-cRNA] complexes was corrected by subtraction of the radioactivity eluted from the [ChH + ^{3}H-SV40-cRNA] hybrids (Figure 2). The thermal stability of the [SV40-DNA + ^{3}H-SV40-cRNA] and [c1-21-DNA + ^{3}H-SV40-cRNA] complexes indicates that in both cells long RNA molecules are involved. On the contrary, since 80% of the radioactivity was eluted by heating at 70 C from normal [ChH-DNA + ^{3}H-SV40-cRNA] hybrids, it appears that normal ChH DNA does not contain long complementary DNA with SV40, but may contain short complementary nucleotide sequences of about 10 to 30 nucleotides (14). This interpretation is based on the assumption that RNase does not introduce nicks into RNA molecules which are hybridized to DNA in perfectly complementary base sequences (15). The profile of thermal elution of ^{3}H-SV40 cRNA from [c1-5-DNA + ^{3}H-SV40-cRNA] hybrids was not the same as that from [SV40-DNA + ^{3}H-SV40-cRNA] hybrids and that from blank filters, but more similar to that from ChH DNA. Thus, under these experimental conditions, the amount of SV40 in C1-5 is below detectability (2 genome equivalent).

^{3}H-labeled cRNA was also synthesized using normal ChH DNA as template

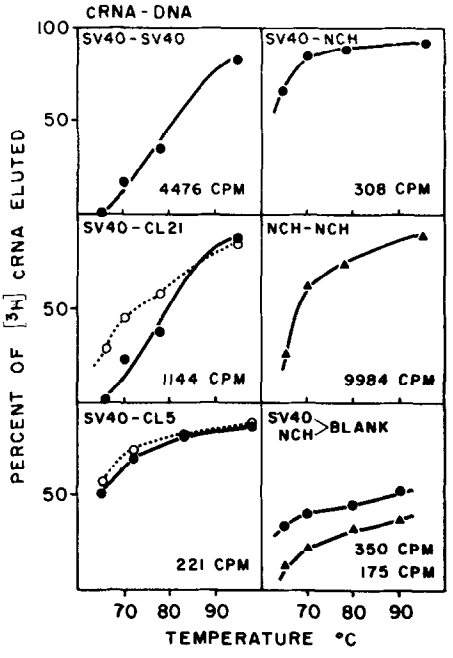

Fig. 2. Thermal elution of ^{3}H cRNA from DNA-RNA hybrid. DNA-RNA hybridization was done as described in Materials and Methods. The input counts of cRNA were: ^{3}H-SV40 cRNA (1.27 X 10^{5} cpm) and ChH cRNA (2 X 10^{5} cpm). The amounts of the immobilized DNA on the filter were: a) SV40 DNA, 0.05 μg; b) C1-21 DNA, 105 μg; c) C1-5 DNA, 148 μg; d) ChH DNA, 195 μg; e) ChH DNA, 74 μg; and f) no DNA.

After RNase treatment, the filter was washed by 2X SSC, put into a vial containing 1 ml of 2X SSC, and incubated for 10 min at the indicated temperature. The eluted radioactivity from the filter was counted. The radioactivity still bound to the filter at 95 C was 5 to 17% of the total. The solid line (●——●) on charts b and c indicate that the percentage of the eluted radioactivity from the filter (0-----0) was corrected by subtraction of the radioactivity eluted from the [ChH-DNA + SV40-cRNA] hybrid at the corresponding temperature.

The radioactivity shown on each chart (a-f) is total radioactivity before heat treatment.

*The background radioactivity was subtracted.

**The radioactivity of [^{3}H-SV40-cRNA + ChH-DNA] hybrid was subtracted.

and the E. coli DNA-dependent RNA polymerase. This RNA hybridized with normal ChH DNA. These RNA-DNA hybrids showed different heat stability from that of [SV40-DNA + ^{3}H-SV40-cRNA] hybrids and [ChH-DNA + ^{3}H-SV40-cRNA] hybrids.

Heat stability of DNA-DNA hybrids. To further investigate the problem of

homology between SV40 DNA and normal ChH DNA, DNA-DNA hybridization was used. It was found that the ^{3}H-labeled SV40 DNA was hybridized with normal ChH DNA, as well as CV-1 DNA, at a level higher than with blank filter (without DNA), as shown by Aloni et al. (4). However, the heat stability of [^{3}H-SV40-DNA + cell-DNA] hybrids was different from that of [^{3}H-SV40-DNA + SV40-DNA] hybrids and also from [^{3}H-cell-DNA + cell-DNA] hybrids (Figure 3).

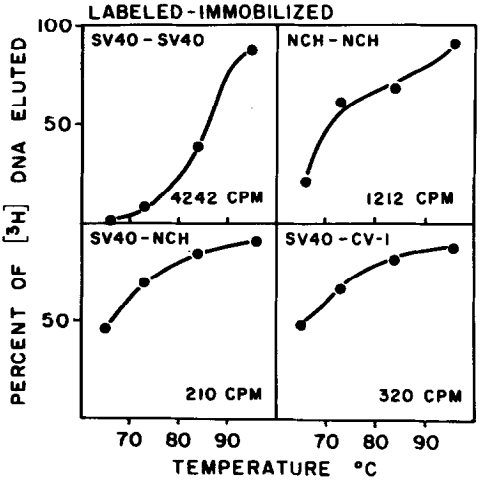

Fig. 3. Thermal elution of ^{3}H -DNA from DNA-DNA hybrid. DNA-DNA hybridizations were carried out as described in Materials and Methods. The amount of DNA on the filter was: a) SV40 DNA, 0.4 μg; b) ChH DNA, 188 μg; c) ChH DNA, 111 μg; d) CV-1 DNA, 148 μg. The input count of ^{3}H-DNA in solution was as follows: ^{3}H-SV40 DNA (8 X 10^{3} cpm, 4.5 X 10^{4} cpm/μg), and ^{3}H-ChH DNA (3.5 X 10^{4} cpm/μg).

The experiment of thermal elution of ^{3}H-DNA from DNA-DNA hybrid was done in the same way as in Figure 3. The radioactivity shown on each chart is total radioactivity before heat treatment. The background was not subtracted from this value.

DISCUSSION

These experiments indicate that, by the criterion of thermal stability of the [SV40-cRNA + cell-DNA] hybrids, the normal ChH DNA, and possibly CV-1 DNA, do contain some short homologous nucleotide sequence with the SV40 DNA, possibly as many as 10-30 nucleotides. Experiments

with DNA-DNA hybridization also favor such an interpretation. This background does interfere with a quantitative analysis of the SV40 DNA integrated into the DNA of the corresponding SV40-transformed cells. However, if the [DNA + ^{3}H-RNA] hybrids on the filter are incubated at 70 C for 10 min after RNase treatment, then the short complementary cRNA, as well as non-specific binding to membrane, is eliminated. Thus, if the number of SV40 genome equivalents is calculated only on the basis of ^{3}H-cRNA radioactivity remaining on the filter after heating at 70 C, then the Cl-21 cells contain between 20-30 genome equivalents per cell, while the Cl-5 contain less than 2 genome equivalents per cell. This low number of viral genome equivalent integrated into cell DNA is in accordance with estimates reached in other laboratories by different techniques with other SV40-transformed cells (16,17).

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