SYMMETRIC TRANSCRIPTION OF SIMIAN VIRUS 40 DNA IN THE NUCLEI OF TRANSFORMED MOUSE CELLS

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SUMMARY

The transcription pattern of SV40 DNA in the transformed mouse 3T3 cell line SV101 has been determined by DNA-RNA hybridization studies using the separated strands of restriction endonuclease derived viral DNA fragments as molecular probes. RNA isolated from the cytoplasm of these cells contained asymmetrically transcribed base sequences complementary to only the early region of the viral genome, while RNA isolated from purified nuclei contained symmetrically transcribed sequences complementary to all regions. The presence of late, anti-early, and anti-late RNA copies in the nuclei of these transformed cells, and their absence from the cytoplasm, suggests that RNA processing and/or transport are significant control mechanisms in the expression of this eukaryotic DNA.

INTRODUCTION

The cytoplasm of monkey cells productively infected with SV40 contains asymmetric viral specific RNA that is predominantly complementary to the early and late strand regions of the viral genome (see Fig. 1), while the nuclei of such cells contain viral specific RNA that appears to be symmetrically transcribed and has been shown to be complementary to at least the early, late, and anti-early viral DNA regions (2); as expected, this nuclear viral RNA can form RNA-RNA duplexes (3, 4), an occurrence that can confuse the quantitation of specific RNA sequences obtained from DNA-RNA hybridization studies. The cytoplasm of mouse cells transformed by SV40 also contains asymmetric viral specific RNA, but in contrast to productive infection,

Abbreviation : SV40, Simian Virus 40

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transformed cell cytoplasmic viral RNA is generally complementary to only the early strand region (5). Although the transcription pattern of SV40 DNA in the nuclei of such transformed cells has not been reported, the fact that viral RNA extracted from whole cells contains substantial but variable amounts (depending on the strain) of late or anti-early (5) and anti-late (6, 7) sequences suggests that some degree of symmetric transcription also occurs in this system. In this communication we report the results of a direct analysis of viral transcripts present in both the cytoplasm and nuclei of an SV40 transformed mouse cell line (SV101) as determined by DNA-RNA hybridizations using the separated strands of restriction endonuclease derived viral DNA fragments. These results indicate that extensive symmetric viral DNA transcription does indeed occur in the nuclei of such cells, and that selective processing and/or transport determines the nature of transcripts ultimately detected in the cytoplasm.

MATERIALS AND METHODS

Cells. SV101 cells, a transformed mouse line (8), containing about 8 copies of SV40 DNA per diploid quantity of cell DNA (6, 9), were grown in Corning plastic roller bottles in Dulbecco's modified Eagle's medium (Gibco) containing 10% calf serum under an atmosphere of 5% CO_2.

Separation of nuclei and cytoplasm. Nuclear and cytoplasmic fractions of SV101 cells were prepared by a modification of the procedure of Wu and Zubay (10). Specifically, confluent cells were harvested by scraping, washed with cold isotonic buffer (30 mM Tris-HCl, 120 mM KCl, 5 mM MgAc, 0.1 mM dithiothreitol, pH 7.5), suspended in cold hypotonic buffer (10 mM Tris-HCl, 1 mM KCl, 3 mM MgAc, 0.1 mM dithiothreitol, pH 7.5), and then disrupted with a Dounce homogenizer. The disrupted cell suspension was centrifuged at 2000 rpm for 5 min and the supernatant used for extraction of cytoplasmic RNA. The nuclear pellet was washed four times with isotonic buffer containing 0.1% Triton X-100 (Sigma) and eventually washed and resuspended in isotonic buffer before extraction of nuclear RNA.

Preparation of RNA. Cytoplasmic RNA was prepared by the method of Perry et al. (11), and nuclear RNA was prepared as described by Soeiro and Darnell (12). Approximately 2.6 mg of cytoplasmic RNA and 0.34 mg of nuclear RNA were obtained from 10^9 cells, and it is assumed that only 0.001-0.002% of the total RNA was SV40 specific (13).

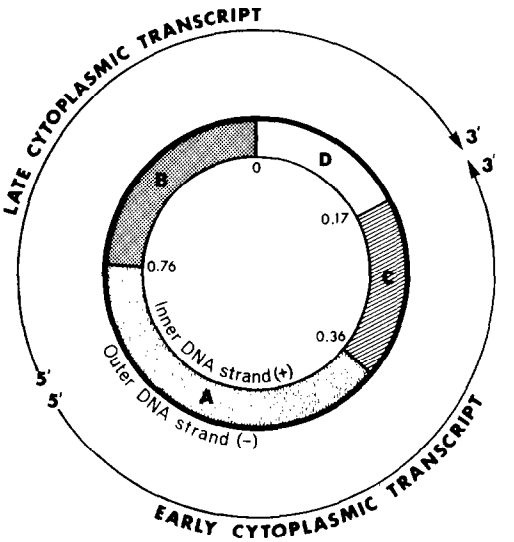

FIG. 1. Schematic diagram of double stranded SV40 DNA showing the combined Hpa 1 and Eco R1 restriction endonuclease fragments. The dark outer DNA strand (often referred to as the minus (-) or early (E) strand) is the template for early lytic cytoplasmic RNA, while the light inner DNA strand (referred to as the plus (+) or late (L) strand) is the template for late lytic cytoplasmic RNA. The numbers on the inner strand indicate the standard map positions (1) for the restriction endonuclease sites recognized by Eco R1 (0) and Hpa 1 (0.17, 0.36, 0.76); the areas A, B, C, and D represent the double stranded fragments produced by cleavage with these enzymes. The individual strands of a given restriction endonuclease fragment can be identified by the same plus or minus designations as the original intact strands, and the terms "anti-early" and "anti-late" are conveniently used to specify those nucleotide sequences that are not complementary to cytoplasmic transcripts. The map regions corresponding to early and late lytic cytoplasmic transcripts are also indicated in this figure (from about 0.67 to 0.17 units), and it should be noted that the combined action of Eco R1 and Hpa 1 results in fragments A, C and B, D that closely resemble the early and late regions, respectively.

Preparation of ^{32}P-labeled separated strands of restriction endonuclease digested SV40 DNA. ^{32}P-labeled circular covalently closed SV40 DNA, prepared as previously described (14), was digested with Eco R1 and/or Hpa 1 restriction endonucleases (Bethesda Research Laboratories, Inc.), and the resulting fragments separated by gel electrophoresis through 1.4% agarose (15, 16). The DNA bands in sliced gels were then dissolved in 5 M $NaClO_4$, 50 mM Tris, pH 7.4, and double stranded DNA recovered by hydroxylapatite chromatography (17). Unit length SV40 linear molecules (Eco R1 digestion product), or specific restriction endonuclease fragments, were denatured in alkali, hybridized to an excess of SV40 specific complementary RNA (transcribed only from the minus (E) strand), synthesized in vitro using E. coli RNA polymerase (17), and separated into early (E) and late (L) DNA strands by hydroxylapatite

chromatography using the purification procedure described by Sambrook et al. (16, 17).

RNA-DNA hybridization. The hybridization of unlabeled nuclear or cytoplasmic RNAs to the ^{32}P-labeled separated strands of intact or fragmented SV40 DNAs was performed in solution (0.15 ml) at 68°C, and assayed by hydroxylapatite chromatography, according to the procedure of Khoury et al. (18). Background values for the percent label behaving as double stranded material in the absence of added RNA were subtracted from the results of each RNA-DNA hybridization assay, and ranged from 0.5% in the case of fragment B (+) to 14% in the case of fragment D (-). Boiling of RNA preparations in 0.3 M NaOH for 10 min prior to the hybridization reaction reduced the percent label behaving as double stranded material to background levels, demonstrating that the hybridization was specific for RNA and not due to possible contamination of the RNA preparations with SV40 DNA present in the transformed cells.

RESULTS

The results of a direct analysis of the SV40 RNA transcripts present in the nuclei or cytoplasm of SV101 transformed cells are shown in Figs. 2 and 3. Although the nuclei of these cells contain extensive amounts of RNA molecules complementary to all of the separated strands of the resolved restriction endonuclease fragments derived from combined Eco Rl/Hpa 1 digestion of SV40 DNA, the cytoplasm contains molecules that are predominantly complementary to the early region of the minus (E) strand (see Fig. 1). Due to our experimental result (data not shown) that in vitro synthesized SV40 complementary RNA could only drive 72% (background subtracted) of the entire minus (E) strand into a hybrid, instead of a theoretical 100%, we do not feel confident in analysing the data presented in Figs. 2 and 3 quantitatively; such an analysis would also be impaired, in the case of nuclear RNA, by competing RNA-RNA self reassociations. In any event, significant amounts of RNA complementary to all of the eight separated strands of SV40 DNA exist in the nuclei of SV101 cells (Fig. 2), and it appears that RNA species complementary to the early region of the minus (E) strand (the region specifying cytoplasmic viral RNA) represent the most abundant class.

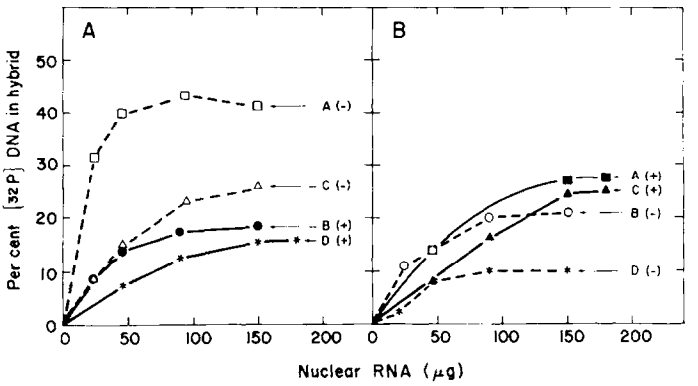

FIG. 2. Hybridization of ^{32}P-labeled separated strands of SV40 DNA restriction endonuclease fragments with unlabeled nuclear RNA. Hybridization analyses were performed as described in Materials and Methods, and background values for the self reassociation of the DNA probes have been subtracted. The nature of the ^{32}P-labeled fragments used (A (-), C (-), B (+), D (+), A (+), C (+), B (-), and D (-)), are indicated in the figure, and correspond to the strands shown schematically in Fig. 1. Note that all fragments hybridize to some extent with nuclear RNA. About 0.7 ng of DNA were used per assay.

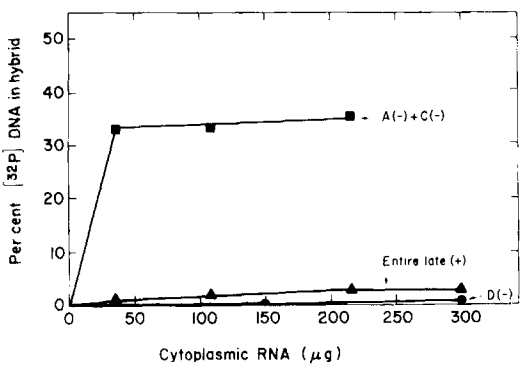

FIG. 3. Hybridization of ^{32}P-labeled separated strands of SV40 DNA restriction endonuclease fragments with unlabeled cytoplasmic RNA. Hybridization analyses were performed as described in Materials and Methods, and background values for the self reassociation of the DNA probes have been subtracted. The nature of the ^{32}P-labeled fragments used (a mixture of A (-) and C (-), the entire (+) strand, and D (-)) are indicated in the figure, and correspond to the strands shown schematically in Fig. 1. Note that only the mixture of A (-) and C (-) fragments hybridize significantly to cytoplasmic RNA. About 0.7 ng of DNA were used per assay.

DISCUSSION

The existence of symmetric transcripts in the nuclei of cells productively infected (2-4) or transformed (this paper) by SV40, and their absence from the cytoplasm of these cells, indicates the involvement of selective processing and/or transport in the expression of viral information. Transcription complexes (viral nucleoprotein complexes) isolated from the nuclei of productively infected cells also catalyze extensive symmetric RNA synthesis in vitro (19). It therefore appears that non-selective transcription is an inherent property of viral RNA synthesis in the nucleus, and need not be due to the suggested ability (6, 7, 20) of host cell promoters to fortuitously "read-through" integrated viral DNA copies known to be present in transformed cells, and suspected to be present in productively infected ones (21). Instead, the symmetric copying of complementary DNA strands could be the normal mechanism of viral transcription in this eukaryotic system, whether in transformed or productively infected cells.

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