The size distribution of nuclear and cytoplasmic virus-specific RNA molecules in rat embryo cells transformed by Adenovirus type 2

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SUMMARY

Nuclear and cytoplasmic RNAs in rat embryo cells transformed by adenovirus type 2 (Ad 2) were isolated and fractionated by sucrose density gradient centrifugation. Size distribution of virus-specific nuclear and cytoplasmic RNA molecules was examined by hybridizing fractionated RNA with Ad 2 DNA. The results showed that virus-specific RNA species in the nucleus are heterogeneous in size with sedimentation values of 7–45 S. On the other hand, virus-specific RNA in the cytoplasm appears to be homogeneous with a main peak of approximately 20 S. These findings together with the previous results[4,17,18] suggest that cytoplasmic virus-specific RNA is produced by specific cleavage of larger heterogeneous nuclear RNA containing virus-specific portions and non-viral portions of different sizes.

INTRODUCTION

Virus-specific RNA was synthesized in rat embryo cells transformed by adenovirus type 2 (Ad 2) which produced no infectious virus[1,2]. Only 5 to 10% of the whole viral genome is transcribed[3]. Virus-specific RNA sequences are synthesized in the nucleus, and most, if not all, of nuclear virus-specific RNA molecules are transported into the cytoplasm[4]. Virus-specific RNA was found on the polyribosome[5], and was shown to contain a polyadenylic acid sequence[5]. Therefore, it may be said that virus-specific RNA posseses several typical characters of messenger RNA (mRNA) in eukaryotic cells. In eukaryotic cells, high molecular weight heterogeneous nuclear RNA (HnRNA) containing messenger RNA sequences are synthesized in the cell nucleus, and only small portions of HnRNA are transported

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into the cytoplasm after the cleavage of large molecules[6,7,8]. We report here size distributions of virus-specific RNA molecules in the nucleus and in the cytoplasm of Ad 2 transformed rat embryo cells, together with those of cellular HnRNA and cytoplasmic RNA.

MATERIALS AND METHODS

Cell and lebeling of RNA

Rat embryo cells transformed by Ad 2 (8617)[1] were grown in suspension in Eagle's minimum essential medium with 10% calf serum to 1×10^6 cells/ml. ^{3}H-Uridine (22.7 C/mM, New England Nuclear, 8 μCi/ml) was added and cells were incubated further for 6 hr at 37°. Cells were collected by centrifugation at 1,200 rpm for 10 min. and washed with cold phosphate-bufferd saline.

Separation of nuclear and cytoplasmic fraction

Labeled cells were suspended in hypotonic buffer [0.01 M NaCl, 0.01 M Tris-HCl, 0.0015 M $MgCl_2$, pH 7.4] containing 0.2% Nonidet-P 40 and the nuclei and cytoplasms were separated as described previously[4].

Extraction of nuclear RNA

Nuclei were digested with RNase-free DNase (Worthington Biochemical Co.) in high salt buffer and RNA was isolated by extraction with hot phenol and phenol-$CHCl_3$ as described[9]. Specific activity of [^{3}H] RNA was 5.9×10^4 cpm/μg.

Cytoplasmic RNA

Cytoplasmic RNA was purified by the hot phenol-SDS method[10]. Specific activity of [^{3}H] RNA was 5.2×10^4 cpm/μg.

Treatment of RNA with DMSO

Nuclear RNA was precipitated with 2 volumes of ethanol and dissolved with small volume of 0.1 × SSC and DMSO (dimethyl sulfoxide, Nakarai Chemicals) was added to come to final concentration of 95% and incubated for 30 min. at 37°[16]. After dialyzing against 0.1 × SSC (SSC ; 0.15 M NaCl + 0.015 M Na_3 Citrate) for 1 hr at room temperature, RNA was precipitated with 2 volumes of ethanol, and dissloved in 0.1 × SSC.

DNA preparation

Adenovirus DNA was extracted from purified virion by the method of Green and Pina[11]. Cell DNA was puified by Marmur's procedure[12]. DNA–RNA hydridization was performed by the membrane filter method as described[13].

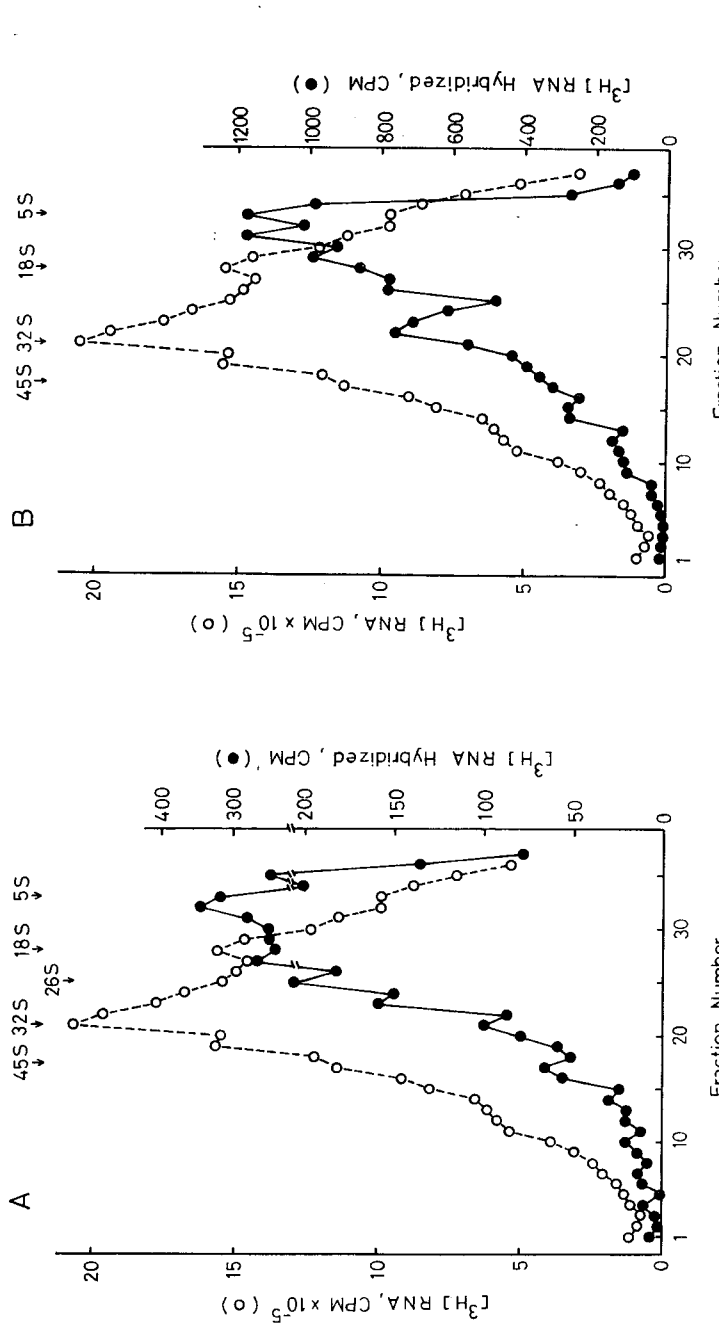

Fig. 1. Sedimentation profile of DMSO treated nuclear [^{3}H] RNA from Ad 2 transformed cells and profile of RNA species hybridizable with Ad 2 DNA or cellular DNA. Nuclear [^{3}H] RNA (3.4×10^7 cpm) was centrifuged in a 15 to 30% sucrose density gradient for 16 hr at 19,000 rpm at 20°. 5 μl of each fraction was precipitated by cold TCA and counted by liquid scintilation counter. After the addition of 50 μl of 20×SSC (SSC: 0.15 M NaCl+0.015 M Na_3 citrate) to each fraction (0.45 ml) hybridization was carried out for 20 hr at 66° with Ad 2 DNA (1 μg/filter) (A) and with Ad 2 transformed cell DNA (5 μg/filter) (B). Then, DNA filter was treated with 20 μg/ml of RNase at 30° for 1 hr, and the radioactivity was determined as described. Back ground (cpm bound to empty filter) was subtracted. Total nuclear RNA treated with DMSO ○– –○, RNA hybridized to DNA ●——●.

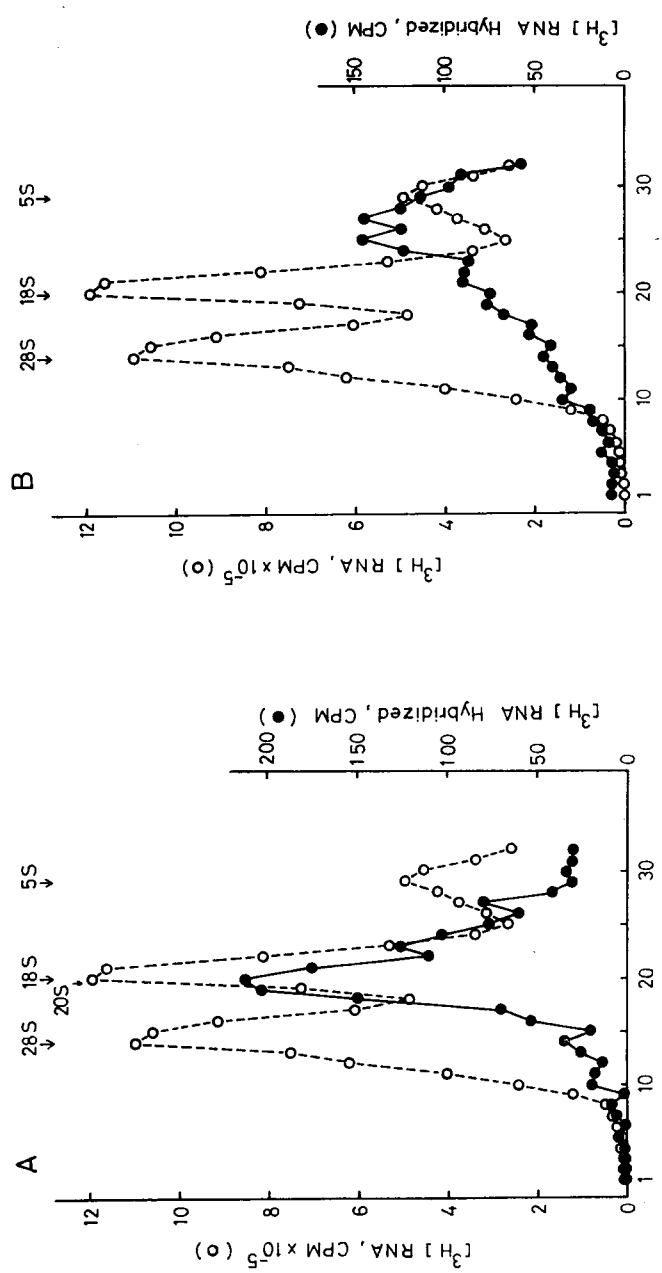

Fig. 2. Sedimentation profile of cytoplasmic RNA from Ad 2 transformed cells and RNA species hybridizable with Ad 2 DNA or cell DNA. Cytoplasmic [^{3}H] RNA (2.1×10^7 cpm) was centrifuged in a 15 to 30% sucrose density gradient for 16 hr at 24,000 rpm at 20°. After the addition of 15 μl of 20×SSC (SSC: 0.15 M NaCl+0.015 M Na_3 citrate) to each fraction (0.135 ml), hybridization was carried out with Ad 2 DNA (1 μg/filter) (A) and with Ad 2 transformed cell DNA (5 μg/filter) (B) as described in the legend of Figure 1. Total cytoplasmic RNA o---o, RNA hybridized to DNA ●——●.

Fractionation of RNA by sedimentation in sucrose density gradients

Nuclear RNA was centrifuged in a 15 to 30% sucrose density gradient in SDS buffer [0.01 M Tris-HCl pH 7.4, 0.01 M NaCl, 0.5% SDS] with Hitachi RPS 25 rotor at 19,000 rpm for 16 hr at 20°.

Cytoplasmic RNA was 24,000 rpm for 16 hr with Hitachi RPS 65 rotor at 20° in a 15 to 30% sucrose density gradient in SDS buffer.

RESULTS

Size distribution of virus-specific RNA and cellular RNA in the nucleus

Nuclear RNA was isolated from Ad 2 transformed rat embryo cells labeled with [^{3}H]-Uridine (6 hr), and treated with DMSO to disrupt aggregated RNA molecules. Figure 1 shows the sedimentation patterns of DMSO treated RNA in sucrose density gradient and profiles of RNA species hybridizable to Ad 2 or Ad 2 transformed rat embryo cell DNA. Labeled nuclear RNA sediments with a main peak of 32 S, is typical for ribosomal RNA precursor (broken line)[14]. The pattern of virus-specific RNA, hybridizable to Ad 2 DNA, showed a heterogeneous size distribution with sedimentation values of 7–45 S, and RNA species larger than 26 S were found (Fig. 1. A). A similar size distribution is also observed for cellular RNA hybridizable to cell DNA (Fig. 1. B). Because low concentrations of cell DNA (5 μg) and a rather short incubation time in the hybridization reaction with cell DNA were used for the detection, RNA molecules hybridizable to cell DNA probably mainly represent those transcibed from repetitive cell DNA sequences[15], and do not contain virus-specific nucleotide sequences because of the presence of only a small copy number of the viral genome in Ad 2 transformed cell DNA[23]. The DMSO treatment of RNA preparation eliminates the possibility of the presence of the aggregates among RNA molecules, and the existence of large virus-specific RNA molecules as a heterogeneous population can be concluded. These results agree with the previous reports of Parsons and Green[16] and R. Wall *et al*[5].

Virus-specific RNA and cellular RNA in cytoplasm

In contrast to the heterogeneous size distribution of virus-specific RNA in the nucleus, cytoplasmic virus-specific RNA was found to be rather homogeneous on polyacrylamide gel electrophoresis[18]. An Essentialy similar pattern was obtained by sucrose density gradient centrifugation as shown in Fig. 2. A, indicating the presence of major 20 S species.

On the other hand, cell RNA hybridizable to cell DNA showed a heterogeneous size distribution, and its low hybridizability possibly reflects a rather rich population of mRNA species transcribed from unique DNA

sequences (Fig. 2, B).

DISSCUSION

The results reported here indicate that high molecular weight HnRNA contains virus-specific sequences in nuclei of adenovirus type 2 transformed cells. If only 10% of the viral genome is transcribed in Ad 2 transformed cells, at most 1.2×10^6 daltons of RNA which corresponds to 24 S could be transcribed, but RNA species larger than 26 S corresponding to over 1.4×10^6 daltons were found in the nucleus. This suggests the presence of RNA molecules covalently linked with viral RNA and cellular RNA. Tsuei *et al*[17]. reported evidence supporting the above view ; RNA molecules hybridized with Ad 2 DNA on nitrocellulose membrane filters were eluted without RNase treatment and it was shown that such RNA has nucleotide sequences homologous to normal and transformed cellular repetitive DNA.

Virus-specific RNA molecules in the cytoplasm were detected as homogeneous RNA species of major 20 S species, of which the molecular weight corresponds to 0.95×10^6 daltons. This result is similar to the previous report of R. Wall *et al*[5]. who showed that adenovirus-specific polyribosomal RNAs from Ad 2 transformed cells have a major peak of 20 S and minor peaks of 26 S and 16 S on sucrose density gradient centrifugation. In our experiment, minor virus-specific RNA species of 26 S and 16 S were not clearly detectable, possibly due to their low quantities in the cytoplasm but such RNA species were found when whole cell RNA extracted by the hot phenol-SDS method was used (data are not shown). In eukaryotic cells, mRNA molecules are transcribed from chromosomal DNA as a part of high molecular weight heterogeneous nuclear RNA molecules which contains repetitive and unique RNA sequences in the same molecule and only unique RNA sequences are proceseed into cytoplasmic RNA[19,20,21,22]. In a similar manner, virus-specific RNA molecules are transcribed from multiple copies of partial viral genome integrated in cellular chromosome[23,24], and most probably are present initially in the nucleus as large heterogeneous molecules containing different sizes of non-viral portions[5,14], followed by specific cleavage of molecules during transportation into the cytoplasm.

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