POLY A SEQUENCE IN VIRUS-SPECIFIC RNA FROM HUMAN ADENOVIRUS TRANSFORMED CELLS[1]

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Abstract: Virus-specific RNA was isolated from cells transformed by human adenovirus 2 by two cycles of hybridizations and elutions with adenovirus 2 DNA. Viral RNA was resistant to pancreatic and T_1 RNase digestion and could bind to Millipore filter in high salt buffer. We suggest that viral RNA molecules from transformed cells contain poly A sequence.

INTRODUCTION

DNA tumor viruses (SV40, polyoma and adenovirus, etc.) can cause lytic infection with production of infectious progeny and death of infected cells. They also can cause temperate infection, in which a fraction of infected cells may become transformed and no infectious virus produced. In these transformed cells, virus DNA is thought to be integrated into host chromosome and replicates as part of the hereditary material of cells. It is well known that viral DNA is transcribed because viral mRNA can be indentified by DNA-RNA hybridization (Green, 1970).

We have previously isolated virus-spesific RNA from cells transformed by human adenovirus 2 and 7 by multiple hybridization with and elutions from homologous viral DNA molecules purified by this selection procedure hybridized with both viral DNA and DNA from untransformed normal cells. We suggested that RNA molecules containing covalently linked viral and cellular sequences transcribed in human adenovirus transformed cells (Tsuei *et al.*, 1972; Green *et al.*, 1970; Fujinaga *et al.*, 1970). The synthesis of polycistronic RNA species is additional evidence for the integration of viral DNA into host cell genome.

One striking feature of eukaryotic cell RNA is the presence of tracts of polyriboadenylic acid (poly A), about 200 nucleotides, in messenger RNA and heterogeneous nuclear RNA (Edmonds *et al.*, 1971; Lee *et al.*, 1971; Darnell *et al.*, 1971) but not in ribosomal RNA or transfer RNA. Poly A has also been found in the mRNA of vaccinia virus (Kates, 1970), Herpesvirus (Bachenheimer & Roizman, 1972) and adnovirus (Philipson *et al.*, 1971). Here we report that poly A segment is also found in adenovirus specific RNA molecules from tranformed cells.

MATERIALS AND METHODS

Cell culture and virus infection: Adenovirus 2-transformed rat embryo cells (8617) and human KB cells were grown in suspension medium supplemented with 5% horse serum. Cultures of KB cells at a density of 3×10^5/ml were infected with 50–100 plaque-forming units of adenovirus 2 per cell (Fujinaga *et al.*, 1968).

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Abbreviations: SSC, Standard Saline Citrate (0.15 M NaCl-0.015 M Trisodium Citrate); SDS, Sodium Dodecyl Sulfate; RNase, Ribonuclease.

DNA

Adenovirus DNA was isolated from purified virus (Green, 1963). DNA from untransformed cells was prepared by either of two methods (Marmur, 1961; Pina & Green. 1969),

RNA

KB cells infected with adenovirus 2 were labeled with 20 μCi/ml of [^{3}H] uridine or 5 μCi/ml of [^{3}H] adenosine (New England Nuclear Co.) from 2–18 or 16–18 hr after infection. Adenovirus 2 transformed cells were labeled for 8 hr. Cytoplasmic RNA was purified as described before (Parsons & Green, 1971). If necessary, cytoplasmic RNA was precipitated in 0.2 M LiCl to remove small molecular weight RNA species.

DNA-RNA hybridization and elution procedures: Hybridization in the presence of 7.5 M urea at 37°C was used as described (Tsuei *et al.*, 1972). Denatured viral DNA was immobilized on 25 mm nitrocellulose membranes and 6.5 mm circles were made with a paper punch. Filters were placed in 10×65 mm test tube containing labeled RNA, 7.5 M urea, 0.01 M N-Tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid (TES) (Calbiochem Co.), 2×SSC, and 0.05% SDS in a final volume of 150 μl. Annealing was performed at 37°C for 24 hr. Filters were then washed five times with 1 ml of 2×SSC; RNA was eluted with 8.0 M urea, 0.01 M TES, 0.1×SSC and 0.5% SDS at 60°C for 10 min.

RNase treatment: DNA-RNA hybrid was treated with 0.5 ml of pancreatic RNase (20 μg/ml) in 2×SSC at room temperature for 1 hr to remove non-specific binding. For RNase resistant test, RNA samples were treated with combination of pancreatic RNase (20 μg/ml) and RNase T_1 in 2×SSC at 37°C for 30 min (Lai & Duesburg, 1972).

Binding of RNA to Millipore filters: The procedures of Lee *et al.*, (Lee *et al.*, 1971) was used. Samples were diluted into 10 volumes of ice-cold 10 mM Tri (pH 7.6)–500 mM KCl–1 mM $MgCl_2$. After 10 min in cold, they were filtered through Millipore filters (HA 0.45 μm, Millipore Filter Co.) previously soaked in the same solution and the filters were washed twice with 10 ml of this salt solution. Filters were then dried and counted in toluene scintillation mixture.

RESULTS

RNase resistance of viral-cell mRNA from adenovirus 2 transformed cells

1. RNase treatment on viral-cell DNA-RNA hybrid form.

RNA from adenovirus transformed cells contained less than 0.05% virus-specific mRNA. In order to select this viral RNA, [^{3}H] labeled cytoplasmic RNA from transformed rat cells was hybridized with 5 μg per filter adenovirus 2 DNA. Bound RNA was eluted and re-hybridized with fresh DNA filter. After incubation, treatment of pancreatic and T_1 RNase was performed to remove the cellular part of viral-cell mRNA and non-specific bindings. Then it was eluted again and annealed with viral DNA, cell DNA or *E. cloi* DNA (Table 1). Control experiment without RNase treatment was compared. Sample without RNase treatment showed 38% and 25% binding to virus and cell DNA respectively. RNase treated sample showed similar binding. The resistance to RNase when it was in DNA-RNA hybrid form indicated that viral-cell RNA probably had a tail that could not be removed by RNase, and was homologous to cell DNA and contained poly A sequence.

2. RNase treatment on single-stranded viral-cell RNA.

[^{3}H] labeled cytoplasmic RNA from adenovirus 2 transformed cells was preciptited by 0.2 M LiCl overnigh to remove small molecular weight RNA species. It was then hybridized with adenovirus DNA and eluted from DNA-RNA hybrid twice to select viral specific RNA as before. This single-stranded RNA was treated with combination of pancreatic and T_1 RNase at 37°C for 30 min. Incubation mixture was

Table 1. RNase Resistance of Viral-cell mRNA from Ad 2 Transformed Cells

RNA		DNA		Bound Radioactivity	
Sample	Input cpm	Source	μg/Filter	cpm**	%
2 cycle purified, no RNase treatment	1,900	Ad 2	5	723	38
		rat cell	50	487	25.6
		E. coli	50	35	1.8
		none	0	8	0.4
2 cycle purified, + RNase treatment*	2,130	Ad 2	5	720	33.8
		rat cell	50	475	21.3
		E. coli	50	32	1.5
		none	0	11	0.5

* RNase treatment was in 50 units/ml of RNase T_1 and followed by 20 μg/ml of pancreatic RNase in 2×SSC, room temperature for 1 hr.

** Average of duplicate reactions.

Table 2. RNase Treatment on Single Stranded RNA

RNA Sample	RNase Treatment	cpm* Bound	% of Resistance
2 cycle purified Ad 2 RNA (viral-cell RNA)	−	255	100
	+	134	52
Transformed cell cytoplasmic RNA	−	217,010	100
	+	27,955	13.5

* Background subtracted, average of duplicate reactions.

Table 3. Binding of RNA to Millipore Filters

RNA	Treatment	RNA Bound	
		cpm*	% of input
Viral-cell RNA (2 cycle purified)	input	306	100
	high salt	135	44
	low salt	11	3.6
RNA from Ad 2 infected KB cells	input	6.8×10^5	100
	high salt	1.2×10^5	18.4
	low salt	2.0×10^3	0.3

* Background subtracted, average of duplicate reactions.

precipitated by 10% trichloroacetic acid (TCA). Radioactivity was measured on filter by counting in toluene scitillation fluid. Control samples without RNase treatment were standardized as 100%. Two cycle purified adenovirus 2 RNA showed 52% resistance to RNase as compared with transformed rat cell cytoplasmic RNA, from where viral RNA was purified, of 13.5% resistance.

Binding of viral RNA to Millipore filters

It is known that RNA molecules containing poly A segment can bind to Millipore filters under high KCl concentration (Lee *et al.*, 1971). As before, LiCl precipitated RNA from adenovirus 2 transformed rat cells were used for purification of virus specific RNA. Selected viral RNA showed 44% binding to Millipore filters under high salt condition (0.5 M KCl–0.01 M Tris–0.001 M MgCl) and 3.6% under low salt condition (0.12 M NaCl–0.01 M Tris). Whereas adenovirus 2 lytic infected human KB cell cytoplasmic RNA showed only 18% and 0.3% binding under high and low salt condition respectively. The 18% binding might represent some poly A sequences in the adenovirus 2 mRNA in lytic infected cells.

DISCUSSION

In this communication we report the presence of polyadenylic acid (poly A) rich regions in virus specific RNA from human adenovirus 2 transformed rat cells. Our data indicated that this viral RNA is highly resistant to pancreatic and T_1 RNase digestion, no matter it is treated as single stranded or as DNA-RNA hybrid form. This RNA could bind to Millipore filters in 0.5 M KCl high salt buffer but not in 0.12 M NaCl buffer. These unique properties are commonly used for the recongnition of poly A segments.

The poly A regions appear to be linked to the remainder of RNA molecules (Edmonds *et al.*, 1971) and usually located at 3′ OH end of RNA. Most poly A tracts are large, about 150–200 nucleotide residues in length. Whether the poly A is transcribed from genome or added after transcription is controversial. In the case of vaccinia virus, the poly A is copied from poly T stretches of vaccinia DNA (Kates, 1970). But Philipson *et al.*, (1971) have shown that adenovirus DNA contains no sequence complementary to poly A and it is added to mRNA after transcription. In the case of adenovirus transformed cell RNA, the poly A could be formed in the following two possibilities: (i) The viral genome is incorporated into repetitive sequence of cell chromosome and T-rich region of the cellular repetitive sequence is transcribed. (ii) Poly A is added post-transcriptionally during RNA transportation from nucleus to cytoplasm. The origin of poly A in viral RNA from transformed cells is still an open question.

The function of poly A in eukaryotic cell mRNA is not clear. It may be involved in the regulation of translation or in processing of heterogenous nuclear RNA. The finding of poly A in tumor virus RNA provides another exciting approach to the viral oncogenesis.

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