COMPARISON OF SEQUENCE HOMOLOGY OF POLY(A) AND NON-POLY(A) CONTAINING 34S RNA OF AKR MURINE LEUKEMIA VIRUS

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Received November 23,1976

SUMMARY:

AKR MuLV 70S RNA was separated on Poly(U)-Sepharose into poly(A) and non-poly(A) containing 34S subunits. The ratio of the two fractions was 2:1, respectively. Both fractions were hybridized to AKR MuLV [^{3}H]cDNA, and the hybrids were assayed by nuclease S_1 and cesium sulfate centrifugation. The poly(A) and non-poly(A) subunits hybridized to [^{3}H]cDNA to the same extent (80%), with identical $CO_{1/2}$ values; and the hybrids of both fractions had identical T_m values (81°C in 0.15 M NaCl). These results demonstrate that the poly(A) and non-poly(A) containing subunits of the AKR genome have identical or very similar base sequences in the heteropolymeric regions.

INTRODUCTION:

The genomes of C-type viruses are composed of a 70S RNA complex containing two to four 30-40S subunits (1-3). Several approaches have been used to study the structure of the 70S complex including kinetic inactivation analysis (4-6), molecular complexity experiments (7,8), electron microscopy (9,10), nuclease T_1 fragment analysis (11,12) and sedimentation analysis (13,14). Some of these studies suggested that the 70S complex contained 30-40S RNA with individually unique sequences (a haploid genome), whereas most of the studies suggested sequence homology among the 30-40S subunits (a polyploid genome). In previous studies it was demonstrated that the 70S complex of several RNA viruses contains poly(A) regions (15-20). More recently we have demonstrated that the AKR MuLV 70S complexes, all of which contain poly(A) regions,

Abbreviations: $CO_{1/2}$, the concentration of RNA of 50% hybridization of the cDNA; T_m, the temperature at which 50% of the hybrid is dissociated; MuLV, murine leukemia virus(es); RSV, Rous sarcoma virus.

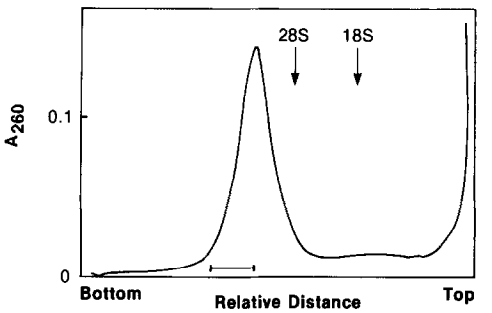

Figure 1. Purification of AKR 34S RNA. 70S RNA was prepared from 3 h-harvested AKR virus, heat dissociated to 34S subunits and sedimented in a sucrose gradient (18). The bottom half of the 34S peak was pooled, ethanol precipitated and collected by centrifugation (100,000 x g, 1 h). The 34S RNA was dissolved in 0.5 ml of H_2O and layered on a 12-ml linear sucrose gradient (10-30%) in .01 M Tris^{6} HCl, pH 7.5, .01 M NaCl, .001 M EDTA, .01% SDS, and centrifuged in a Spinco SW41 rotor at 30,000 RPM (110,000 x g) for 17 h at 4°C. The gradient was collected (0.4 ml fractions) and the A_{260} profile followed. Fractions containing the bottom half of the 34S peak (⊢—⊣) were pooled, ethanol precipitated, and dissolved in a minimum volume of hybridization buffer.

can be dissociated and fractionated into 34S RNA molecules: two-thirds of which contain poly(A) and one-third of which does not contain poly(A) (18). In the experiments presented here we have examined the 34S RNA subunits, fractionated on the basis of the presence or absence of poly(A) regions, for sequence homology. The results demonstrate no detectable sequence differences in the heteropolymeric regions of the subunit fractions.

MATERIALS AND METHODS:

Isolation of AKR MuLV 70S RNA. Murine AKR virus was isolated from a spontaneously activated AKR mouse embryo fibroblast line at 3-h intervals and banded on isopycnic sucrose gradients (18). 70S RNA was isolated from purified virus by phenol extraction and sucrose gradient centrifugation (18).

DNA-RNA hybridization. [^{3}H]cDNA was synthesized with the endogenous reverse transcriptase reaction and purified as previously described (21). The [3H]cDNA product had a specific activity of 1-2 x 10^{7} cpm/μg and was totally digested by nuclease S_1. DNA-RNA hybrids were formed in 0.45 M NaCl at 62°C for 48 h and analyzed with S_1 nuclease (21). The percent hybridization is defined as the acid-precipitable cpm resistant to S_1 nuclease divided by the total acid-precipitable cpm.

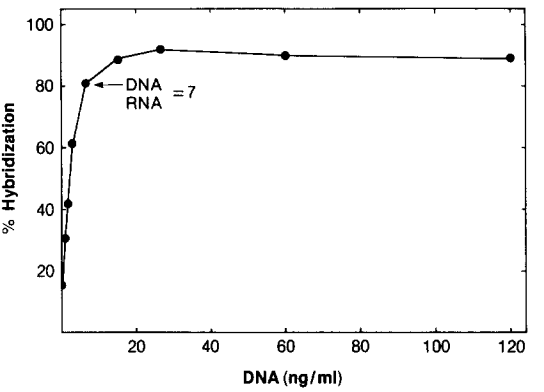

Figure 2. Protection of AKR [^{125}I]RNA with a [^{3}H]cDNA probe. AKR [^{125}I] 70S RNA (5 - 10 x 10^7 cpm/µg) was prepared as described by Woo, et al. (23) and concentrated by ethanol precipitation. The [^{125}I]RNA (2,800 cpm) was hybridized with [^{3}H]cDNA (4 x 10^5 cpm/µg) in 0.45 M NaCl for 48 h. After incubation, an equal volume of .01 M Tris-HCl, pH 7.5, containing 10 µg/ml RNase A and 20 µg/ml ribonuclease T_1 was added and incubated for 1.5 h at 37°C. The [^{125}I]RNA that was nuclease-resistant was determined as in "METHODS".

RESULTS:

To obtain intact subunits, AKR MuLV 34S RNA was purified by two successive sucrose gradient centrifugations. The sucrose gradient profile of the second gradient fractionation is shown in Figure 1 and illustrates that the purified RNA migrates as a symmetrical peak of 32-36S. To reduce low-molecular-weight contamination, only the rapidly sedimenting half of the peak was used in subsequent experiments. The poly(A) and non-poly(A) subunits were fractionated as described (18), except the non-poly(A) and poly(A) fractions were eluted at 20°C and 50°C, respectively, without intermediate temperature elutions. The ratio of poly(A) containing fraction to the non-poly(A) containing fraction was 2:1, and both fractions contained intact 34S RNA as judged by sucrose gradient centrifugation.

To determine the sequence homology between the poly(A) and non-poly(A) subunits, both fractions were hybridized with the [^{3}H]cDNA probe. The properties of the cDNA are shown in Figure 2. As illustrated by the

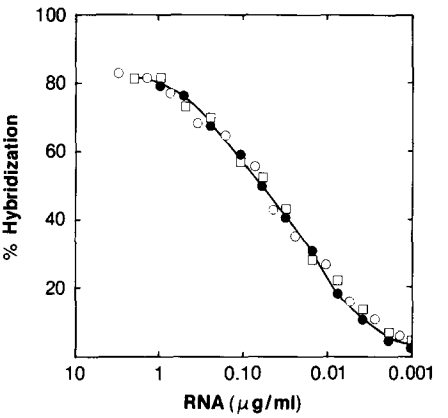

Figure 3. Hybridization of poly(A) and non-poly(A) 34S subunits with [^{3}H]cDNA. 34S RNA fractions were hybridized to [^{3}H]cDNA as in "METHODS". The symbols are ● , non-poly(A) containing RNA; □, poly(A) containing RNA; ○, mixture of poly(A) and non-poly(A) containing RNA (2:1).

results, at a DNA:RNA ratio of 7:1 approximately 90% of [^{125}I] 70S RNA can be protected from ribonuclease digestion. These data suggest that the cDNA used in these experiments is representative of the entire genome. When the 34S RNA fractions were hybridized with this cDNA, approximately 80% of the cDNA hybridized to either fraction with a $Co_{1/2}$ of 0.06μg/ml (Figure 3). When both fractions were mixed and hybridized to [^{3}H]cDNA, no increase in hybridization was detected. Therefore, both fractions contain similar sequences that represent most or all of the AKR genome.

To confirm the hybridization data obtained with S_1 nuclease assays, DNA-RNA hybrids were formed with saturating levels of both fractions and analyzed by cesium sulfate centrifugation. As illustrated in Figure 4, unhybridized single-stranded [^{3}H]cDNA banded at about 1.4 g/cc. Hybridization of the [^{3}H] probe with the poly(A) and non-poly(A) subunits shifted 75-80% of the [^{3}H]cDNA to a density of 1.5 - 1.7, the expected density for viral RNA and RNA-DNA hybrids. As above, hybridization with the combined fractions did not increase the yield of hybrids.

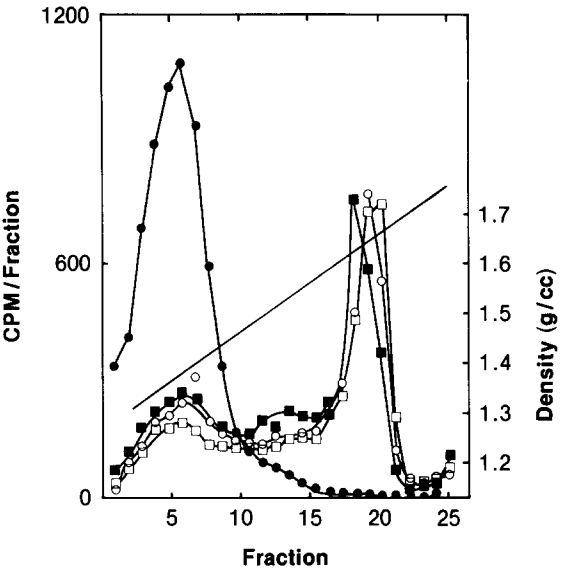

Figure 4. Cesium sulfate density gradient analysis of DNA-RNA hybrids. [^{3}H]cDNA-RNA hybrids were prepared with an excess of 34S RNA. Hybrids (4,700 cpm) in 0.11 ml were layered on a 5-ml Cs_2SO_4 solution (1.52 g/cc) in 0.05 M Tris HCl, pH 7.5 and centrifuged at 34,000 rpm for 72 h in a Spinco SW50.1 rotor at 5°C. Fractions (0.2 ml) were collected, mixed with 2.0 ml of H_2O and counted in Aquasol. The density of Cs_2SO_4 was determined from refractive index measurements. Symbols are ○, non-poly(A) RNA; ■, poly(A) RNA; □, poly(A) and non-poly(A) RNA (2:1); and ●, [^{3}H]cDNA incubated without RNA. Recoveries were greater than 95%.

The base sequence homology between the two subunit fractions was further analyzed by determing the thermal stability of the DNA-RNA hybrids (Figure 5). Hybrids formed with the poly(A) and non-poly(A) subunits had identical thermal denaturation curves, with T_m values of 81°C.

DISCUSSION:

The poly(A) and non-poly(A) subunits of AKR viral RNA hybridized to AKR cDNA with identical kinetics and thermal stability. In addition, each fraction and a mixture of both fractions hybridized to the same extent. These results clearly demonstrate that the poly(A) and non-poly(A) subunits have identical or very similar heteropolymeric sequences. These results are in agreement with those of Wang and Duesberg (11) who

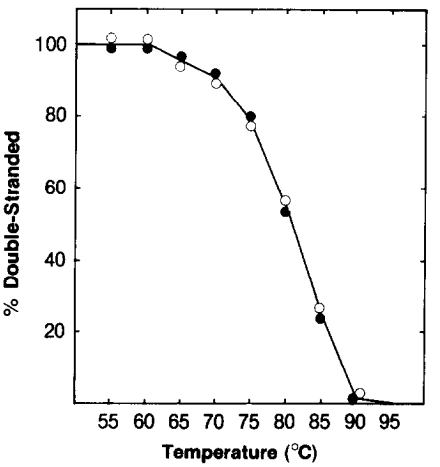

Figure 5. Thermal stability of hybrids. DNA-RNA hybrids were formed with [^{3}H]AKR cDNA and an excess of 34S RNA in 0.45 M NaCl at 62°C for 48 h and diluted threefold with .01 M Tris HCl, pH 7.3. Aliquots (0.05 ml) were heated at the indicated temperature for 10 min, chilled in ice and treated with S_1 to determine the percentage of double-stranded hybrids remaining. The symbols are ● , poly(A) RNA and ○ , non-poly(A) RNA.

demonstrated that the ribonuclease T_1 fingerprint maps of the RSV poly(A) and non-poly(A) RNA were identical.

The fact that only two-thirds of the 34S RNA subunits are found polyadenylated may be attributed to either *in vivo* processing of the RNA or degradation. Recently King and Wells (22) presented evidence that the non-poly(A) subunits of RSV are due to random degradation of the poly(A) containing subunits. Because of the method we used for isolation of the 34S RNA and the consistency of the observation (18), it is unlikely that our results are due to random degradation of viral RNA. It seems more probable that these results reflect specific adenylation or deadenylation of only part of the 34S subunits. The biological significance of these differences is not known.

Lastly, our findings are consistant with a polyploid model of the viral genome; however, they can not rule out the possibility that the genome is haploid, and each population contains molecules with and without poly(A).

ACKNOWLEDGEMENTS:

The authors would like to thank Barry Lazar for excellent technical assistance with virus production. This research was sponsored by the National Cancer Institute under Contract No. NCI-CO-25423 with Litton Bionetics, Inc.

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