THE SEPARATION OF MOUSE RETICULOCYTE 9S RNA INTO FRACTIONS OF DIFFERENT BIOLOGICAL ACTIVITY BY HYBRIDIZATION TO POLY [U] -CELLULOSE

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SUMMARY. The 9S RNA isolated from mouse reticulocyte polysomes has been separated into two fractions which differ in their adenine-rich stretches and their biological activities. One RNA fraction is eluted from poly[U]-cellulose columns at 4° C. This fraction has only twelve percent of the biological activity for mouse α and β globin synthesis compared to a control 9S RNA sample. The other fraction hybridizes to the poly[U] and is eluted at a temperature of 65° C. This RNA is at least seven times more active than is the RNA eluted at 4° C. These results demonstrate that the fraction of 9S RNA which hybridizes to poly[U]-cellulose contains most of the active globin mRNA.

Polyadenylic acid-rich regions have been found covalently linked to some high molecular weight RNAs (1-4), messenger RNAs (5-12) and viral-specific RNAs (13-17). It is still uncertain whether these adenine-rich stretches are involved in either the processing or the translation of messenger RNAs in eucaryotes.

RNAs containing adenine-rich sequences have been separated from total cellular RNAs by their selective binding at high ionic strength to cellulose covalently linked to oligodeoxythymidylate (oligo[dT]) (18-22) and polyuridylate (poly[U]) (14,23). A fraction enriched in 9S RNA has been purified from rabbit reticulocyte polysomal RNA by its selective binding to poly[dT]-cellulose (20). The 9S RNA fraction isolated from the poly[dT]-cellulose column is active in rabbit globin synthesis. However, there has been no data on whether the adenine-rich stretches in the reticulocyte 9S RNA are necessary for biological activity. Neither has it been shown that the 9S RNA fraction contains RNA species which are not retained by the hybridization procedure used for isolating the "active" messenger RNA.

In these studies, we show the fractionation of mouse 9S RNA, prepared by SDS treatment of polysomes, on poly [U]-cellulose columns. The poly [U]-cellulose columns resolve the 9S RNA into different fractions. These have different capacities for globin synthesis.

Methods - The isolation of mouse reticulocytes, preparation of ribosomes, and the dissociation of ribosomes were carried out as previously described (24-26). Isolation of 9S RNA by zonal ultracentrifugation was by the method of Lingrel et al. (25).

The capacity of each mouse 9S RNA fraction to synthesize mouse α and β globins was measured in a duck reticulocyte lysate system by the method of Lockard and Lingrel (27,28). 0.5 ml cell-free systems were used. After incubation, 9S and control cell-free systems were pooled, the globin was extracted with acid acetone and the mouse α and β globin chains were separated on a column of CM-52 cellulose (28). The fractions were analyzed as previously described (28).

Results

Fractionation of mouse 9S RNA by thermal chromatography on poly [U]-cellulose columns - Columns of poly [U]-cellulose were prepared by the method of Britten (29). The columns were pretreated at 65° C in 10 mM Tris, 100 mM NaCl, pH 7.4, until the 260 nm reading was below 0.002. A column containing one gram of cellulose binds 168 μg of poly [A].

Less than 168 μg of mouse 9S RNA in 1 ml of 100 mM NaCl, 10 mM Tris, pH 7.6, were applied to the column at 4° C. 1 ml fractions were collected and the absorbance at 260 nm of each was read. The temperature was raised sequentially to 25° C, 45° C, and 65° C and fractions were collected at each temperature as before. The RNA was precipitated from ethanol and taken up in 50 mM Tris, pH 7.4.

The recovery of 9S RNA from each fraction is shown in Table I. Most of the material absorbing at 260 nm elutes from the column at 4° and at 65°. The total optical density at 260 nm recovered from the column is

Table I. Fraction of 9S RNA by Thermal Chromatography on Poly[U]-cellulose Columns

Temperature Eluted	% 9S RNA Eluted at each temperature
4° C	30
25° C	7
45° C	19
65° C	44

The percentages are the average of 3 runs in which 103-121 μg 9S RNA were applied to the column. Percentages are calculated assuming that any poly[U] eluted was in the 65° C fraction.

Table II. Capacity for Globin Synthesis of Mouse 9S RNA Separated by Thermal Chromatography on Poly[U] - cellulose Columns.

Sample	----picomoles leucine incorporated----- α globin	β globin	α + β globin
9S control	180	233	413
4° eluant	15	35	50
65° eluant	163	183	346
9S heated at 65°	183	233	416
9S + 5 g poly U	185	291	476
9S + 5 g poly U heated at 65°	163	241	404

The micrograms of 9S RNA in each sample was calculated by comparing the area under the 9S RNA peak on polyacrylamide gels to that of an unfractionated 9S RNA sample. 3 μg 9S RNA was added to each cell-free system. Cell-free protein synthesis and analysis of fractions were carried out as in Methods.

25-50% greater than that applied, showing that some poly[U] was also being eluted from the column. The poly[U] was most eluted at 65° C, and it accounted for two-thirds or more of the optical density at 260 nm of this fraction (J. G. unpublished observations).

Table II shows the capacity for globin synthesis of the RNAs fractionated at 4° C and 65° C. The RNA eluted at 4° C has less than one-seventh the activity of the RNA eluted at 65° C or of the control sample. In a repeat experiment, the RNA eluted at 25° C had comparable activity to the 4° C sample, whereas the RNA eluted at 45° C was as active as the 65° C sample (results not shown). In order to ensure that neither some poly[U] contamination of the 9S RNA fractions nor heating would inhibit the cell-free systems, several controls were also run. When poly[U] was incubated with 9S RNA for 15 minutes at 4° C, the same amounts of mouse α and β globins were synthesized as in the control 9S sample. Further, the activity of the 9S RNA was not affected by pre-incubating samples at 65° C, both in the presence and absence of poly[U].

Total 9S RNA migrates as three distinct bands on 6% polyacrylamide gels (30). Preliminary results on 6% polyacrylamide gels suggest that the RNA eluting at 4° C migrates as two distinct bands which correspond to the two heavier bands in the total 9S RNA. The RNA eluting at 65° C migrates as two diffuse bands, one in the 12S region and one migrating just ahead of the two major bands in the total 9S RNA. The contamination with poly[U] so far precludes any correlation of these bands with the total 9S RNA fractions (experiments in progress).

Discussion - 9S RNA fractions isolated from rabbit and duck reticulocyte polysomes have been shown to contain adenine-rich sequences (5,9,11); in mice, the hybridization of 9S RNA to DNA is caused by hybridization of short poly[A] regions in the 9S RNA to complementary regions in the DNA (31).

In this report, the hybridization of adenine-rich regions in the mouse 9S RNA to poly[U] has been used to separate the 9S RNA into two fractions. The fraction having adenine-rich sequences too short to hybridize to poly[U] is only one-seventh as active in globin synthesis as

that which elutes from the poly[U] column at 65° C. There are several possible explanations for these results.

(1) The 65° C fraction contains all the globin messenger RNA: the fraction eluting at 4° C may have residual activity due to a small amount of messenger RNA failing to hybridize to the poly[U] on this run, most of the material in this fraction being breakdown products of ribosomal RNA. Although there is some preliminary evidence from acrylamide gel analysis suggesting the main optical density bands in other reticulocyte 9S RNAs are the α and β globin chains (32), analysis of isolated bands of mouse 9S RNA for globin synthesis does suggest that at least one of the main bands is a ribosomal RNA breakdown product (33). Competition hybridization of methylated mouse 9S RNA with ribosomal RNA also indicates that the 9S RNA fraction contains some ribosomal RNA products (31). The isolation of globin mRNA uncontaminated by other RNA species is important for more detailed studies on the messenger RNA translation, for studies on sequence and secondary structure.

(2) Globin messenger activity is related to the length of the adenine-rich region in the mRNA. It has been suggested that the adenine-rich regions in the globin mRNA are necessary for translation (5) perhaps by a ticketing mechanism (34) or by changes in secondary structure exposing initiation sites or conferring ribonuclease resistance on the RNA. The RNA failing to hybridize to the poly[U] might therefore be globin mRNA deficient in poly[A] regions and therefore inactive in globin synthesis. Analysis of the different RNA fractions for poly[A] regions is in progress.

(3) The fraction eluting at 4° C may contain messenger RNA which has long adenine stretches, but these are buried in the secondary structure. This might preclude binding to the poly[U]-cellulose and also account for the very low biological activity.

(4) It is also possible that the results are due to artifacts

such as a binding of oligo[U] stretches to the mRNA eluting at 4° C, thus rendering the previously active mRNA inactive in a cell-free system. However, the experiments on the effect of poly[U] on the translation of 9S RNA indicate this explanation is unlikely.

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