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Characterization of Short Time Labeled Adenosine Monophosphate-Rich Ribonucleic Acids of Soybean[1]

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ABSTRACT

The total population of newly synthesized ^{32}P-AMP-rich RNA has been separated into two major types based on repeated fractionation on methylated albumin-kieselguhr columns. The purified D-RNA which elutes, under our experimental conditions, primarily in the salt gradient has a GMP/AMP ratio of about 0.8 and an AMP + UMP content of about 56 mole per cent. The purified TB-RNA which preferentially remains bound to the column in the salt gradient has a GMP/AMP ratio of about 0.4 to 0.45 and an AMP + UMP content of about 65 mole per cent. In addition to being distinguished by their fractionation on the methylated albumin-kieselguhr column and base composition analysis, purified D-RNA and TB-RNA have different size distributions on sucrose gradient and acrylamide gel fractionation, are differentially associated with polyribosomes and have different stabilities in the tissue.

In some early studies on RNA metabolism in plants (13) we described an RNA fraction of soybean which was labeled with ^{32}P-orthophosphate at a rate of least twice that of rRNA, which was polydisperse sedimenting in the range of 10S to greater than 50S on sucrose gradient analysis, which had a short half-life relative to rRNA and which had a base composition giving a GMP/AMP ratio of about 0.6 similar to the dGMP/dAMP ratio of soybean DNA. This fraction of RNA was termed DNA-like or D-RNA[4] based on the similarity to soybean DNA in base composition and because similar rapidly labeled RNAs in animal cells have been referred to as D-RNA (4, 37). A relatively small fraction of the D-RNA was isolated in association with polyribosomes (26). Since soybean D-RNA was described (13), a large number of laboratories have described similar types of rapidly labeled, AMP-rich RNAs in plants (3, 9, 16, 17, 20, 28, 39). In addition, Ellem and co-workers (7, 8) showed that a significant amount of rapidly labeled RNA of animal cells was not eluted from MAK columns by the salt gradient. This type of RNA which remains tightly bound to MAK columns is present in a number of plant systems (9, 16, 17, 39).

In this paper we report on the further characterization of AMP-rich RNAs of soybean, and to a lesser extent carrot, and show that there are at least two distinct classes of AMP-rich RNAs present in short time labeled ^{32}P-RNA. These can be distinguished from each other and from precursors to rRNA based on fractionation on the MAK column, base composition analysis, acrylamide gel fractionation, and sedimentation on sucrose gradients. Preliminary evidence indicates that these two types of AMP-rich RNA have very different half-lives and are differentially associated with polyribosomes.

MATERIALS AND METHODS

Soybean seedlings and carrot discs were prepared as previously described (13, 20). The tissue slices were incubated in 50 μg/ml chloramphenicol and after a preincubation of 2 hr, unless otherwise stated, were labeled with carrier-free ^{32}P-orthophosphate (usually 2 mc per 10 g tissue in 20 ml). RNA was extracted as previously described (13, 21). Acrylamide gel fractionation of the RNA was accomplished using the methods of Loening (29). MAK columns were prepared and run essentially as described by Mandel and Hershey (30), except that albumin with a higher degree of methylation was used with different salt gradients. Details of experiments will be presented with the data.

After the salt gradient was completed, the RNA remaining bound to the MAK column (crude TB-RNA) was eluted by "stripping" the column with 0.5% SLS. The bulk of the RNA was eluted in a 15 ml volume after a bed-volume of SLS was passed through the column. About 2 mg of carrier rRNA were added to the 15 ml eluate, and sodium acetate was added to a final concentration of 0.15 M. An equal volume of phenol containing hydroxyquinoline and *m*-cresol was added (21). After vigorous agitation, the sample was centrifuged at 15,000 rpm for 15 min in a Sorvall type 34 rotor. The RNA was precipitated from the aqueous layer by addition of 2.4 volumes of ethanol or pelleted overnight in a Spinco type 40 rotor.

The RNA eluting in the D-RNA region of MAK columns was collected by sedimentation in a Spinco type 40 rotor overnight together with carrier rRNA. The pelleted RNA was dissolved in 0.5% SLS containing 0.15 M sodium acetate and precipitated by addition of 2.4 volumes of ethanol.

The crude D-RNA or TB-RNA samples were then subjected to rechromatography on MAK columns for two additional cycles. After three cycles through the MAK column, the purified D-RNA and TB-RNA samples were fractionated by acrylamide gel electrophoresis and sucrose gradient centrifugation.

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[4] D-RNA and TB-RNA are used only to identify the fractions off MAK columns which elute after rRNA in the salt gradient and in SLS, respectively. Purified D-RNA and TB-RNA are used to identify the two classes of purified AMP-rich RNA as exemplified by D-RNA and TB-RNA after three cycles through the MAK column of Tables II and III and Figures 3 and 4; MAK: methylated albumin-kieselguhr; FU: 5-fluorouracil; G/A: GMP/AMP ratio; SLS: sodium lauryl sulfate.

Base composition analyses were made of the D-RNA and TB-RNA fractions from each of the three cycles of MAK column fractionation. The analyses were made on KOH hydrolysates (0.3 M for 18 hr at 37 C followed by addition of perchloric acid to pH 3.5) of the RNA by electrophoretic separation of the four nucleotides (2 hr; 1000 v, pH 3.5 ammonium acetate buffer). The nucleotides were located using a UV lamp; the four quenching areas were cut out and added to scintillation vials containing 10 ml of the toluene-PPO-dimethyl POPOP solution. The base composition of each sample was calculated as mole per cent from the distribution of ^{32}P in the four nucleotides (13).

RESULTS

In most of the experiments which follow, FU was used to depress rRNA synthesis resulting in the bulk of the ^{32}P-RNA being of the AMP-rich type (17). The FU treatment causes this pattern of RNA synthesis without impairing many biological functions which are dependent upon continued RNA synthesis in several plant systems which have been studied (2, 18, 19, 20, 27).

The primary fractionation of the D-RNA from the 25S rRNA was accomplished as illustrated in Figure 1. As the 18S rRNA was eluting (arrow off) the salt gradient was maintained at that concentration (about 0.9–0.95 M NaCl) until the 25S rRNA was eluted (arrow on); then a new linear salt gradient was initiated. In this way the normal shoulder in the 25S

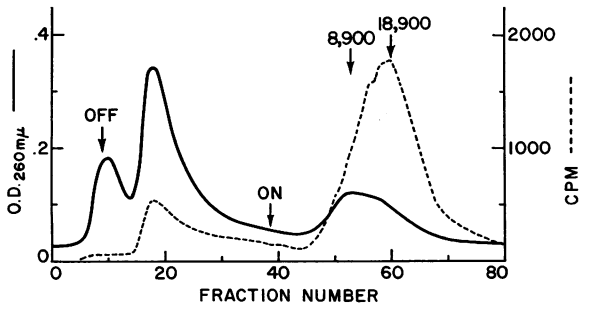

FIG. 1. MAK column fractionation of ^{32}P-D-RNA. The crude D-RNA fraction from 5-FU-treated tissue was refractionated with carrier rRNA. After sample addition and washing of the column with 0.7 M NaCl, a linear 0.7 to 1.2 M (250 ml each) NaCl gradient was initiated. At the "off" position elution with the "mixing" chamber concentration of NaCl (about 0.9–0.95 M) was continued until the "on" position in the profile was reached. At that point a new gradient of 0.9 to 1.2 M NaCl (150 ml each) was initiated.

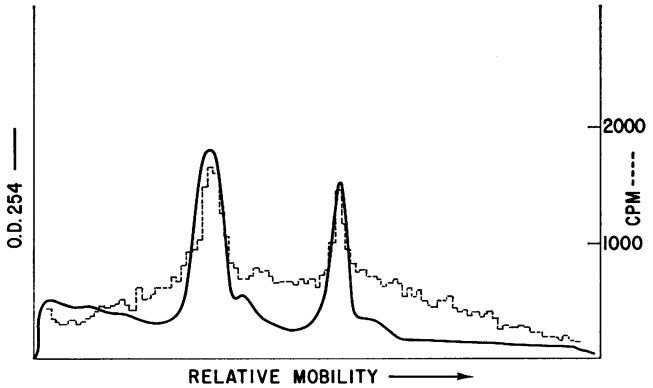

FIG. 2. Acrylamide gel fractionation of the crude ^{32}P-TB-RNA. The ^{32}P-TB-RNA fraction from 2 hr-labeled control tissue was fractionated with carrier rRNA on 2.4% gels at 6 ma/gel for 3 hr.

Table I. *Distribution of ^{32}P-RNA from the Crude D-RNA and TB-RNA Fractions Following Recycling on MAK Columns*

Data are averages of three experiments.

Fraction Added To Column	D-RNA	TB-RNA	Total Recovery
	%		
D-RNA No. 1	76.8	23.2	90
D-RNA No. 2	79.3[1]	20.7	87
TB-RNA No. 1	35.7	64.3	86
TB-RNA No. 2	26.8	73.2[2]	91

[1] This fraction corresponds to D-RNA (No. 3) of Table II.
[2] This fraction corresponds to TB-RNA (No. 3) of Table II.

region (17), which represents primarily aggregated 18S and 25S rRNAs on an absorbance basis (14), and the ^{32}P-labeled D-RNA were separated from the majority of the 25S rRNA. Additionally, this fractionation shows that the ^{32}P-D-RNA is not specifically associated with the major portion of the shoulder of aggregated rRNA as has been suggested by some investigators (16); rather the ^{32}P-labeled D-RNA appears to be associated with the trailing absorbance component of this fraction (note specific activity at absorbance peak and count peak). Normally when crude D-RNA was to be purified by refractionation on MAK columns, a 1.2 M NaCl wash was added at the "arrow on" to elute the crude D-RNA fraction in a small volume. The TB-RNA fraction was then eluted with 0.5% SLS.

Data presented in Figure 2 show that the crude TB-RNA fraction of 2 hr-labeled control tissue (*i.e.*, without FU) contains considerable 18S and 25S rRNAs in addition to the polydisperse AMP-rich RNA. About 15% of the rRNA remains bound to the MAK column under our experimental conditions. The depression by FU of total ^{32}P-labeled crude TB-RNA (17) results primarily from the inhibition of rRNA accumulation in the fraction. So base composition analyses of the crude TB-RNA fraction from control tissue would be significantly affected by contaminating rRNA (which has a G/A ratio of about 1.25) until further purified.

The data of Table I show the redistribution of crude D-RNA and TB-RNA on subsequent refractionation on MAK columns. The D-RNA from column 1 (D-RNA No. 1) redistributed with about 75% eluting in the D-RNA region (D-RNA, No. 2) with salt while about 25% remained as TB-RNA requiring SLS for elution. Of the 75%, about 80% eluted in the D-RNA region (purified D-RNA, No. 3) and about 20% as TB-RNA on the third MAK cycle. The TB-RNA from column 1 redistributed with about 35% eluting as D-RNA and 65% as TB-RNA (TB-RNA, No. 2). Of the 65% eluting as TB-RNA, about 25% eluted in the D-RNA region and 75% as TB-RNA on the third MAK cycle (purified TB-RNA, No. 3). Thus the ^{32}P-RNA originally eluting as D-RNA continues to preferentially elute with salt in the D-RNA region while the TB-RNA fraction continues to preferentially remain bound to the MAK column and to require SLS for elution; there appears to be no complete resolution of these fractions on MAK columns, but the redistribution data of each fraction suggests that the major ^{32}P-RNA component of each fraction differs significantly from the other.

Base composition analyses of the D-RNA and TB-RNA fractions from the MAK columns, which gave the ^{32}P-RNA distributions reported in Table I, are presented in Table II. The D-RNA fraction reached a constant base composition after two cycles through the column with about 56 mole per cent

Table II. *Base Compositions of D-RNA and TB-RNA Fractions off MAK Columns after Two and Three Cycles Through the Column*

The base compositions are based on the distribution of ^{32}P among the four nucleotides from KOH-hydrolyzed RNA which were separated by paper electrophoresis. The numbers adjacent to D- and TB-RNA refer to the number of cycles through the MAK column. D- and TB-RNA No. 3 are referred to as purified D- and TB-RNA in the text. Data are averages of three experiments.

	Mole %				G/A
	C	A	G	U	
D-RNA No. 2	21.3	29.6	23.0	26.1	0.78
D-RNA No. 3	20.3	29.3	23.4	26.0	0.80
TB-RNA No. 2	17.9	39.1	19.5	23.5	0.50
TB-RNA No. 3	17.2	40.7	19.0	23.1	0.47

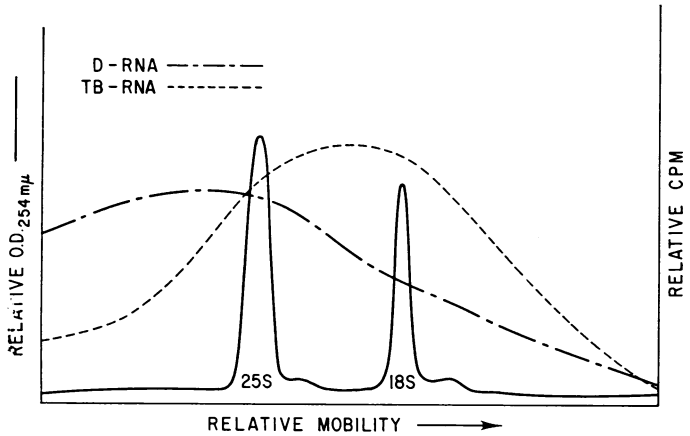

FIG. 3. Acrylamide gel fractionation of purified D-RNA and TB-RNA. Samples of D- and TB-RNA purified through three cycles of MAK column fractionation (samples corresponding to No. 3 from Table II) were fractionated by acrylamide gel electrophoresis as described in Figure 2. The count profiles are representative of a composite of three independent fractionations of three different preparations of purified D- and TB-RNA without considering minor slice to slice variation (100 slices of 0.8 mm each were counted per gel).

A + U and a G/A ratio of about 0.8. The TB-RNA fraction appears to be approaching a constant base composition after three cycles through the MAK column reaching a G/A ratio of about 0.45 to 0.50 for purified TB-RNA.

The purified ^{32}P-labeled D-RNA and TB-RNA samples (*i.e.*, after three cycles through the MAK column) were fractionated by polyacrylamide gel electrophoresis (Fig. 3) and sucrose gradient centrifugation (Fig. 4). Both samples gave a gel profile distribution of great heterogeneity relative to molecular size, but the purified D-RNA was of a larger average molecular size. As on gels, the purified D-RNA sedimented on sucrose gradients with values ranged from about 10S to greater than 60S, with some ^{32}P-RNA being pelleted. On the other hand the purified TB-RNA sedimented over a much narrower range with a rather broad band peaking at about 16S to 18S. As with base composition analyses and MAK column fractionation, the purified D- and TB-RNAs represent different populations of RNA molecules based on acrylamide gel and sucrose gradient fractionation.

Base composition analyses (Table III) were made of sucrose gradient fractions of purified D- and TB-RNA corresponding to the letters shown in Figure 4. The D-RNA fractions gave similar base compositions independent of the molecular size, with values for AMP ranging from 29.5 to 30.7 giving G/A ratios of 0.74 to 0.79. The TB-RNA compositions indicate that the fractions collected from the gradient are somewhat different with a decreasing AMP content as the mean molecular weight increases. This result would be expected if the purified TB-RNA fraction is slightly contaminated with D-RNA (*i.e.*, RNA having a 30% AMP content and a G/A ratio of 0.75–0.80).

The data of Table IV show that carrot D- and TB-RNAs purified by fractionation through two MAK column cycles are similar in base composition to purified D- and TB-RNA of soybean. The two fractions of carrot RNA are also clearly different based on their base composition analyses.

In order to evaluate the relative contribution of D-RNA and TB-RNA (defining these components as those fractions purified through three cycles of MAK columns and having G/A ratios

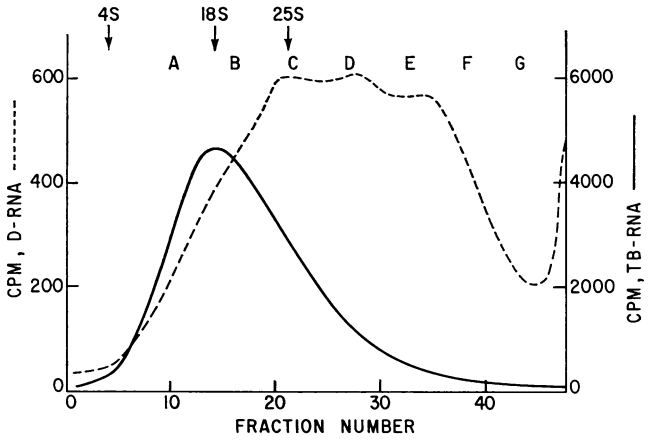

FIG. 4. Sucrose gradient fractionation of purified D-RNA and TB-RNA. Samples of D- and TB-RNA as described in Figure 3 were fractionated on 5 to 20% linear sucrose gradients containing 10 mM sodium acetate buffer, pH 6. Centrifugation was for 12 hr at 23,000 rpm in a Spinco type SW25 rotor. One-half ml fractions were collected. Gradients using different buffers and various additives yielded results similar to those reported for the acetate-buffered gradients.

Table III. *Base Composition of Purified D-RNA and TB-RNA Fractions from Sucrose Gradients*

D- and TB-RNA (No. 3, Table II) were fractionated by sucrose gradient centrifugation and the letters correspond to those fractions noted in Figure 4.

RNA Fraction	Mole %				G/A
	C	A	G	U	
D-RNA	21.2	29.9	22.3	26.6	0.75
A	20.1	29.5	23.2	27.2	0.79
B	20.2	30.2	23.3	26.3	0.77
C	21.2	30.0	23.5	25.3	0.78
D	20.3	29.9	23.7	26.1	0.79
E	21.2	30.6	23.0	25.2	0.75
F	20.6	30.7	22.9	25.9	0.74
G (pellet)	21.6	29.9	22.3	26.2	0.75
TB-RNA	17.8	41.7	17.7	22.8	0.42
A	16.1	44.5	17.9	21.8	0.40
B	16.9	41.3	18.2	22.2	0.44
C	18.4	38.0	19.8	23.5	0.52

Table IV. *Base Composition of Purified ^{32}P-RNA Fractions of Carrot*

The carrot D- and TB-RNA samples were purified through only two cycles of MAK fractionation. Carrot discs were excised and incubated as previously described (14).

RNA Fraction	Mole %				G/A
	C	A	G	U	
rRNA (18S + 25S)	22.9	25.3	31.2	20.6	1.23
D-RNA	21.1	31.6	23.7	23.5	0.75
TB-RNA	18.0	38.3	21.7	22.0	0.57

Table V. *The Time-Course Distribution of the Total AMP-rich RNA between D-RNA and TB-RNA*

Total RNA was extracted from FU-treated excised soybean hypocotyl after the indicated times in ^{32}P-orthophosphate following a 2-hr preincubation. The samples of RNA from duplicate experiments were fractionated on MAK columns as described (17). The base compositions and total ^{32}P-RNA in the crude D-RNA and TB-RNA fractions were determined. From the G/A ratios of each fraction, relative to the G/A ratios of purified D-RNA and TB-RNA (No. 3, Table II), and the total cpm in each fraction, the per cent of the total ^{32}P-AMP-rich RNA present as D-RNA (or TB-RNA) was calculated. In order to make this calculation, the assumption must be made that the total ^{32}P-AMP-rich RNA is composed of a mixed population of ^{32}P-RNA molecules either of the D-RNA type or of the TB-RNA type (No. 3, Table II).

Hr in ^{32}P	G/A		% Total ^{32}P-AMP-rich RNA	
	D-RNA	TB-RNA	D-RNA	TB-RNA
½	0.60	0.42	28	72
1	0.64	0.45	45	55
2	0.75	0.54	58	42
4	0.80	0.58	68	32
6	0.78	0.63	75	25

of about 0.8 and 0.4, respectively) to the total AMP-rich RNA population, RNA was extracted from tissue labeled with ^{32}P-orthophosphate from 30 min to 6 hr. The RNA was fractionated on MAK columns, and the base compositions of the crude D- and TB-RNA fractions were determined. The G/A ratios are reported in Table V. There is a progressive increase in G/A ratio of each fraction with increasing label time. Based on the G/A ratios of purified D- and TB-RNA (Table II, No. 3), the 30 min label yields a crude D-RNA fraction which represents about a 1:1 mixture of each type while the crude TB-RNA fraction represents largely the TB-type. By 6 hr, the crude D-RNA fraction represents ^{32}P-RNA primarily of the D-RNA type while the crude TB-RNA fraction represents close to a 1:1 ratio of each type. Using these values (*i.e.*, the G/A ratio of each fraction) and the total amount of ^{32}P-RNA in each fraction, the percentage of D-RNA and TB-RNA (equivalent to purified D- and TB-RNA after three cycles through the MAK column) at each time was estimated (Table V). There is a progressive increase in the proportion of D-RNA (and a corresponding decrease in TB-RNA) with increasing time of ^{32}P-incorporation from 25 to 30% at 30 min up to about 75% at 6 hr. These results imply that the TB-RNA has a shorter half-life than D-RNA.

Although "chase experiments" are practically impossible to perform in tissues of this type (13), attempts were made in this direction. The tissue was labeled with ^{32}P-orthophosphate for 30 min; the tissue was then surface washed and placed in a 10 mM KH_2PO_4 (pH 6.0) buffer containing 10 μg/ml actinomycin D. Under these conditions ^{32}P-RNA accumulates at a steadily decreasing rate for about 1 hr followed by a decrease in total ^{32}P-RNA (13). The amount of ^{32}P-RNA eluting in the D- and TB-RNA regions from MAK columns and the respective base compositions were determined. The data were sufficient only to conclude that the TB-RNA has a half-life of 1 hr or less while the D-RNA has a half-life of about 4 hr. The values certainly are adequate to distinguish between D-RNA and TB-RNA. The composite value from these experiments for D- and TB-RNA is in agreement with the value previously reported for the total AMP-rich RNA of soybean (13).

Preliminary results on the association of D- and TB-RNA with polyribosomes are presented in Table VI. It was previously shown that a relatively small amount of the AMP-rich RNA (D-RNA) of soybean was polyribosome-associated (26). The data of Table VI show that the major proportion of the AMP-rich RNA associated with polyribosomes is of the purified D-RNA type. Certainly the polyribosome-associated ^{32}P-RNA is greatly enriched in D-RNA relative to TB-RNA compared to the total ^{32}P-AMP-rich RNA.

DISCUSSION

The data presented here show that there are at least two distinct types of rapidly labeled, AMP-rich RNAs in soybean

Table VI. *Association of AMP-rich RNA with Polyribosomes*

Soybean hypocotyl was labeled for 45 min after a 6-hr preincubation in the presence of 50 μM 2,4-D and FU. 2,4-D was used in these experiments in order that a much larger percentage of the ribosomes would be present as polyribosomes. 2,4-D alters the distribution of ^{32}P-RNA present as D- or TB-RNA when compared to control tissue (see Table V). ^{32}P-RNA was prepared from total tissue and from polyribosomes isolated from comparable tissue; the RNA was then fractionated on MAK columns, and the total ^{32}P-RNA (cpm) and base composition analyses were made on the crude D-RNA and TB-RNA fractions. The crude TB-RNA fraction from polyribosomes clearly contains RNA representative of D-RNA (No. 3, Table II) and not of TB-RNA (No. 3, Table II). Further the distribution of the polyribosome AMP-rich RNA between the crude D- and TB-RNA fractions is that expected of purified D-RNA (see Table I). The base composition is reported for polyribosome-associated ^{32}P-RNA of carrot discs following a 20 min exposure to ^{32}P-orthophosphate at which time all of the ^{32}P-RNA is of the polydisperse type (14), and clearly representative of purified D-RNA.

Tissue	AMP-rich RNA	Mole %				G/A
		C	A	G	U	
	%					
Soybean hypocotyl						
Total tissue						
D-RNA	63	19.2	32.3	23.3	25.1	0.72
TB-RNA	37	19.4	34.9	22.2	23.8	0.64
Polyribosomes						
D-RNA	75	20.6	30.3	24.4	24.8	0.81
TB-RNA	25	20.4	29.6	24.8	25.4	0.84
Carrot root						
Polyribosomes		19.1	30.3	24.6	25.3	0.81

(and in carrot). Purified D-RNA has the following properties: (*a*) the base composition gives an A + U value of about 56 mole per cent and a G/A ratio of about 0.8; (*b*) the half-life is about 4 to 5 hr; (*c*) the size distribution is very heterogeneous in sedimentation on sucrose gradients and acrylamide gel fractionation, (*d*) a few per cent is associated with ribosomes in the polyribosome structure; (*e*) it is labeled with ^{32}P-orthophosphate at a greater rate than rRNA but more slowly than TB-RNA. Purified TB-RNA has the following properties: (*a*) the base composition gives an A + U value of about 63 to 65 mole per cent and a G/A ratio of about 0.4 to 0.45; (*b*) the half-life is shorter than that of D-RNA, being about 1 hr (or less); (*c*) it is heterogeneous in size but has a smaller mean size distribution than purified D-RNA; (*d*) it appears not to be polyribosome-associated in significant amounts; (*e*) it is labeled with precursor at about three times the rate of D-RNA labeling.

While purified soybean (and carrot) D- and TB-RNA are similar to corresponding fractions of short time labeled ^{32}P-RNA from other plant tissues (9, 16, 39), there are some differences. These differences may well be the result of the fact that what we are referring to as D-RNA and TB-RNA are those fractions of ^{32}P-RNA which are purified through three cycles of MAK column fractionation to, or approaching, homogeneity in base composition, while this was not done in most other studies.

The purified TB-RNA of soybean appears similar to that reported for pea by Ewing and Cherry (9) where purification was extensive but different from that reported for pea by Johri and Varner (16) except for their 1 hr label data and by Tester and Dure (39) for cotton where either little if any additional purification was attempted or where the labeling time was very long. In both cases (16, 39) some ^{32}P-rRNA and D-RNA would be present in the crude TB-RNA fraction and would significantly affect the base composition analyses. In addition, based on our results, the proportion of D-RNA to TB-RNA by 16 hr would lead to a crude TB-RNA fraction similar to the purified D-RNA of soybean as observed by Johri and Varner (16). There is a large difference in the apparent half-life of D-RNA in pea and soybean. Johri and Varner (16) report a D-RNA (mRNA in their paper) half-life of about 15 min for pea while the value for soybean is certainly in the range of 4 to 5 hr (these half-life values would be at best approximations because of the difficulty in doing chase experiments in plant systems). The only ^{32}P-RNA which is "chased" by ^{31}P-orthophosphate and actinomycin D in our experiments with soybean and carrot in as short a time as reported by Johri and Varner are the precursors to rRNAs (21) which are processed into mature rRNA with a half-life of minutes (21 and unpublished observations). Since precursors to rRNA elute in the D-RNA region of MAK columns and since the column can be run under conditions which cause the bulk of D-RNA to elute as crude TB-RNA, it may well be that these factors account for the apparent short half-life of D-RNA in the experiments of Johri and Varner (16). These half-life differences might also represent species differences. In contrast to soybean, Johri and Varner (16) reported the TB-RNA fraction of peas is more stable (does not chase) than mRNA (D-RNA). Yet in their Figure 8 the TB-RNA fraction appears to chase as fast or faster than their mRNA fraction (corresponding to soybean D-RNA) while in their Figure 9 the TB-RNA continues to accumulate label while the mRNA chases. As pointed out by Johri and Varner (16) the discrepancy between data of Figures 8 and 9 is not clear, nor is the apparent loss of counts from rRNA in their studies. The apparent 4 to 5 hr half-life of purified soybean D-RNA, as reported here, a part of which is polyribosome-associated, is consistent with the rate of decay of protein synthesis (as measured by ^{14}C-amino acid incorporation by tissue slices) and the decay of polyribosomes which show a half-life of about 4 hr in soybean following addition of actinomycin D (unpublished data).

The apparent association of D-RNA (in our experiments) and of RNAs similar in base composition from other systems with polyribosome preparations has been observed in several studies of plant ribosomes (16, 26, 28). The specificity of this association has not been adequately assessed in these studies. While it appears highly probable that the polyribosome-associated D-RNA is in fact mRNA, work with polyribosome-associated, short time labeled RNAs and ribonucleoprotein particles in animal systems (1, 11, 12, 15, 22, 32–34) points out the difficulty in interpreting such data. In addition our data show an enrichment of D-RNA relative to TB-RNA in polyribosome preparations relative to total tissue of both soybean and carrot. The data in fact are suggestive that TB-RNA may not normally be polyribosome-associated. Based on the extraction properties of this AMP-rich RNA (13) and the unique nuclear RNAs of animal cells (4, 36), it may well be that the TB-RNA is in the nuclear fraction in plants. There are several recent reports of adenine-rich clusters in RNAs of animal cells (5, 6, 10, 24, 25, 33). Although the significance of this component is unknown, Sussman (38) recently suggested a role for nontranslated portions of mRNA in controlling the translation process and others have similarly speculated about the function (5, 6, 33). Experiments are underway to evaluate the possible presence of adenine-rich clusters in soybean AMP-rich RNAs by the methods of Lim and Canellakis (24, 25) and of Lee *et al.* (33). Preliminary results indicate the presence of AMP-rich clusters in soybean polyribosomal RNA similar to those reported by Lim and Canellakis (24, 25) for reticulocytes.

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