ON EQUATING LOW MOLECULAR WEIGHT RNA WITH TRANSFER RNA[1]

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Following the discovery of an RNA intermediate in the activation phase of protein synthesis (Holley, 1957; Hoagland et al., 1957), the RNA species involved (sRNA, amino acyl acceptor RNA, here termed "transfer RNA") was purified from a variety of sources and shown to be of low molecular weight, with a sedimentation constant between 4 and 5 S (Osawa, 1960; Klee and Cantoni, 1960; Ofengand et al., 1961). Since that time, reports too numerous to cite have appeared in which it is assumed that any RNA with sedimentation characteristics like transfer RNA can be identified as transfer RNA. We present here data which show this assumption to be erroneous.

Methods: Male rats were given 3 mc of carrier-free ^{32}P intraperitoneally 50 min. before they were killed. Their livers were fractionated into nuclear and cytoplasmic (600 X g supernatant) fractions, using 0.14 M NaCl as the homogenizing medium. This fractionation, as well as the preparation of cytoplasmic RNA and analytical methods not presented in detail, was described earlier (Kenney and Kull, 1963). RNA fractionation by salt precipitation and by chromatography on DEAE-cellulose sheets was based on the methods of Crestfield et al. (1955) and Jacobson and Nishimura (1963).

Results: Analysis of cytoplasmic RNA by sucrose gradient centrifugation shows (Figure 1) that the bulk of the ^{32}P-labeled RNA sediments with the 4 S (Svedberg

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units) peak, as shown previously (Hiatt, 1962; Kenney and Kull, 1963). The experiments that follow were done to determine the extent to which this labeled RNA is transfer RNA.

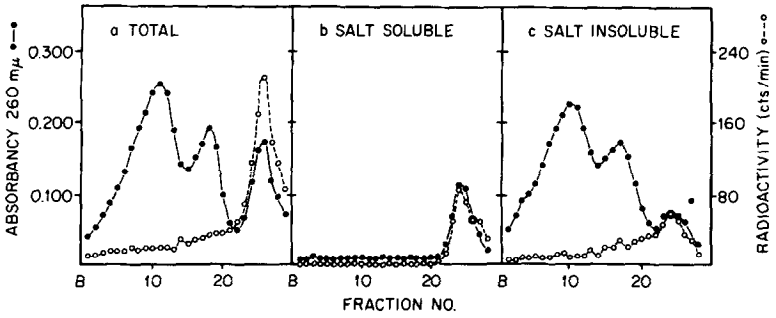

Figure 1. Sucrose density gradient analysis of cytoplasmic RNA and subfractions. Centrifugation was for 3.5 hr. in a 5 to 20% gradient.

The cytoplasmic RNA preparation was brought to 1 M NaCl and left at 3°C overnight. After centrifugation the precipitated RNA (designated as "salt insoluble RNA") was washed once with 1 M NaCl, and this wash was added to the original supernatant. RNA was precipitated from this supernatant ("salt-soluble RNA") by addition of two volumes of ethanol, and both "salt-soluble" and "salt-insoluble" fractions were washed twice with ethanol, then dissolved in 0.01 M Tris pH 7.6 – 0.001 M $MgCl_2$. Analysis of these fractions (Table 1) shows that 12% of the total

Table 1. Salt fractionation of cytoplasmic RNA

RNA fraction	Percent of total		Acceptor activity[2] cts/min/mg RNA
	RNA[1]	^{32}P	
Salt-soluble	12	31	274,000
Salt-insoluble	88	69	6,900

The data on RNA and ^{32}P content are expressed relative to the total unfractionated cytoplasmic RNA. (1) RNA was measured by absorbancy at 260 mμ. (2) Acceptor activity is capacity to form amino acyl-RNA measured in a standard "charging" assay similar to that described previously (Kenney and Kull, 1963). These measurements were made with RNA preparations added at levels wherein total amino acyl-RNA formed is a linear function of RNA added. Reaction mixtures contained 0.2 μc each of ^{14}C-leucine, ^{14}C-isoleucine, and ^{14}C-valine; unlabeled amino acids were omitted.

RNA and 31% of the ^{32}P-RNA are soluble in 1 M NaCl, and that this salt-soluble fraction retains nearly all of the amino-acyl acceptor activity typical of transfer RNA. The RNA of the salt-soluble fraction is almost entirely 4 S (Figure 1), and all the radioactivity of this fraction was associated with the 4 S peak except for a small shoulder of lighter material. Salt-insoluble RNA includes all of the high molecular weight RNA (Figure 1) but retains a diminished 4 S peak containing about 40% of the radioactivity of this fraction.

These experiments provide a strong indication that much of the ^{32}P RNA sedimenting at 4 S is not transfer RNA. Further support for this was sought, employing chromatography on DEAE-cellulose sheets. Transfer RNA is known to migrate on these sheets at salt concentrations above 0.5 – 0.6 M (Jacobson and Nishimura, 1963), and the specificity of this property of sRNA is indicated by the wide use of DEAE chromatography in preparing highly purified transfer RNA. Portions of the RNA fractions described above were pipetted onto 4 x 34 cm strips of DEAE cellulose sheets (Whatman) after first wetting the strips with 0.05 M NaCl. The strips were then irrigated with 0.4 M NaCl to remove ^{32}Pi and other contaminants, then transfer RNA was displaced with 0.7 M NaCl. All the NaCl solutions used in chromatography contained 0.02 M acetate, pH 5.6, and the procedure was carried out at 3°C. After drying, the strips were examined for radioactivity in a 4π strip scanner; representations of the recorded traces are presented in Figure 2.

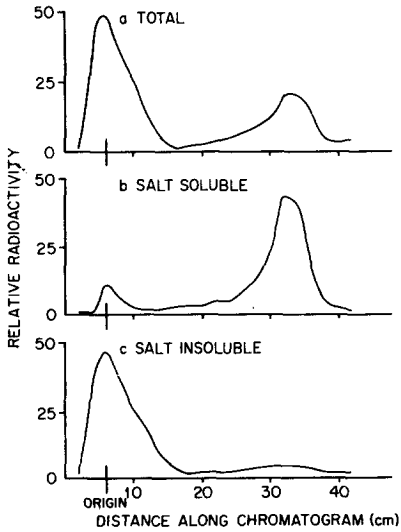

Figure 2.

DEAE-cellulose chromatography of cytoplasmic RNA and subfractions.

Chromatography of the total cytoplasmic RNA yields a result in good agreement with that of salt fractionation, since about 34% of the total ^{32}P-RNA migrated in the fashion expected of transfer RNA. Nearly all of the salt-soluble ^{32}P-RNA was displaced in 0.7 M NaCl; the small amount remaining at the origin may reflect the shoulder of radioactive RNA detected in sucrose gradient centrifugation. A very small portion of the salt-insoluble ^{32}P-RNA moved on the paper, presumably representing the slight contamination of this fraction with transfer RNA that was detected by "charging" assay.

That the material migrating on DEAE-cellulose is, in fact, transfer RNA was confirmed by base composition analysis. The chromatogram of total cytoplasmic RNA (scan represented in Figure 2a) was cut into "origin" and "migrated" regions, and the RNA was eluted from each with 0.5 N KOH. After overnight hydrolysis at 37°C, the nucleotides were chromatographed on DEAE-formate as described by Jacobson (1962). Following elution with NH_4HCO_3, the content of ^{32}P in each nucleotide was determined. The results (Table 2) demonstrate a major difference in the labeling pattern of the two fractions. ^{32}P is distributed in all nucleotides in the "origin" RNA; this distribution is very similar to that previously observed in short-term labeling of total cytoplasmic RNA (Kenney and Kull, 1963). The ^{32}P-RNA that migrated on chromatography is clearly transfer RNA that is labeled exclusively in the —pCpCpA sequence terminating the nucleotide chain. ^{32}P-base composition analysis of the salt-soluble and salt-insoluble fractions yielded results nearly identical to those reported for "migrated" and "origin" RNAs, respectively.

Table 2. ^{32}P-base composition of chromatographic fractions after alkaline hydrolysis

Fraction	Percent of total radioactivity in:			
	Cytidylic	Guanylic	Adenylic	Uridylic
Origin	29	20	27	24
Migrated	70	8	8	14

Discussion: Results of both salt fractionation and migration on DEAE lead to the conclusion that only about 1/3 of the ^{32}P-cytoplasmic RNA is actually transfer RNA, although more than 2/3 of this labeled RNA sediments with the 4S peak on density gradient centrifugation. Therefore, it is clear that the appearance of an RNA in the transfer RNA region of a density gradient (or, by extension, of a methylated albumin column) cannot be taken as identifying that RNA as transfer RNA. A similar conclusion can be derived from the recent report by Rosset et al. (1964) that Escherichia coli ribosomes contain a low molecular weight RNA that differs from transfer RNA.

The nontransfer ^{32}P-RNA found in cytoplasm, largely 4S but partially a mixture of larger RNAs, appears to be synthesized de novo as labeling is distributed throughout all four nucleotides. The origin and nature of this RNA is unknown. In contrast, it is clear that labeling of cytoplasmic transfer RNA is, in these experiments, limited to turnover of the –pCpCpA terminus of the nucleotide chain.

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