Characterization of Transfer RNA Associated with Plasma Membranes

Vincent Joseph Mancusi

The College at Brockport
CHARACTERIZATION OF TRANSFER RNA ASSOCIATED WITH PLASMA MEMBRANES

BY

VINCENT JOSEPH MANCUSI

submitted to the
Department of Biology
State University of New York
College at Brockport
in partial fulfillment of
the requirements for the
degree of Master of Science

June 1976

Drake Memorial Library
State University of New York
Brockport, N. Y. 14420
THESIS DEFENSE

VINCENT MANCUSI

CHARACTERIZATION OF TRANSFER RNA ASSOCIATED WITH PLASMA MEMBRANES

APPROVED

NOT APPROVED

MASTER'S DEGREE ADVISORY COMMITTEE

Larry K. Klein 6/24/76
Major Advisor

Thomas F. Bonner 6/24/76
Committee Member

Robert McLean 6/24/76
Committee Member

Chairman, Graduate Committee

Chairman, Dept. Biological Sciences
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>1</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>2</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>3</td>
</tr>
<tr>
<td>STATEMENT OF PROBLEM</td>
<td>5</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>6</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>10</td>
</tr>
<tr>
<td>RESULTS</td>
<td>18</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>28</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>34</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

I wish to thank Dr. Larry K. Kline for his excellent guidance and supervision of this research project, and also my wife, Judyann and my parents, for without their help and patience this investigation would not have been made possible.
ABSTRACT

RNA extracted from purified rat liver plasma membranes was found to contain transfer RNA. Amino acid acceptor activity was detected for arginine and lysine, a confirmation of previous reports. In addition, acceptor activity was also detected for tryptophan and proline. Isoaccepting tRNA species for lysine and arginine were chromatographed using Benzoylated DEAE-Cellulose column chromatography. No major difference was found between cytoplasmic and membrane isoaccepting species for lysine and arginine transfer RNAs.
INTRODUCTION

The plasma membrane is a complex component covering the surface of the cell. Studies have shown the plasma membrane to have a wide variety of properties; ranging from selective permeability to various ions to possession of receptor sites for hormonal action. The study of the structure and components of the plasma membrane is an active area of research. The Davson-Danielli model represents the plasma membrane structure as a layer of protein which is sandwiched by two layers of phospholipid. Recent studies have demonstrated that the protein molecules may transcend the entire thickness of the membrane. The protein molecules may also have a certain fluidity within the membrane.

Isolation and characterization of plasma membranes have demonstrated the presence of protein, phospholipid, carbohydrate, cholesterol and ribonucleic acid (RNA). The presence of the RNA component of the membrane has been questioned. The criticism centers around the idea that the RNA component is a contaminant from other parts of the cell, i.e. microsomes and/or mitochondria. The criteria for obtaining a totally pure membrane preparation has not been established. The answer to the question as to whether the RNA component is, in fact, a result of contamination or an integral component of the membrane will rest on the purity of the membrane fraction.
Previous studies have indicated that a portion of the RNA associated with membranes is transfer RNA (tRNA). Transfer RNAs have been shown to have isoaccepting species i.e. different molecular species of transfer RNA that accept the same amino acid. For example, tyrosine has three isoaccepting species while phenylalanine has two.

The principal purpose of this investigation was to further detect and characterize transfer RNA associated with the plasma membrane.
STATEMENT OF PROBLEM

RNA may be associated with plasma membranes. The major problem involved in the detection of RNA isolated from plasma membranes centers around the purity of the membrane fraction. The procedures used in this investigation yield a membrane fraction which is at least 95% pure. The molecular species of the RNA component(s) is unknown. Of the three major classifications of RNA species, i.e. transfer, ribosomal, and messenger; transfer RNA is the easiest to detect biochemically, since an aminoacylation reaction is a sensitive and reliable assay procedure.

Previous work has demonstrated that approximately 10-20% of the RNA found in the plasma membrane is transfer RNA. This is based on calculations of tRNA species accepting the amino acids arginine, lysine, and phenylalanine.

The purpose of this investigation is to;
1) Assay for transfer RNA species in rat liver plasma membranes with respect to other amino acids,
2) Chromatographically compare the arginine isoaccepting tRNA species present in both cytoplasmic and plasma membrane fractions, and,
3) Chromatographically compare the lysine isoaccepting tRNA species present in both cytoplasmic and plasma membrane fractions.
LITERATURE REVIEW

An outstanding characteristic of cells is the property that some internal metabolic reactions as well as protection from the external environment is regulated by membranes. Internal membranes function to separate organelles within the cell from the cytoplasmic matrix, i.e. mitochondria and chloroplasts and plasma membranes serve to separate the cell from its external environment. Both types of membranes are primarily lipoprotein in nature.

Membranes of cells generally appear as a homogeneous structure covering the entire cell. When cells differentiate to form tissues variations often result in the structure of the plasma membrane. Desmosomes, tight junctions and gap junctions are all modifications displayed by the plasma membrane when cells differentiate and tissue formation occurs.(1) The phospholipid and protein components of the plasma membrane have been firmly established.(2) In addition, an RNA component has been detected by a number of investigators.(3-6)

Many difficulties are encountered in attempting to isolate a morphologically pure membrane fraction, and as a result, the proof of RNA component(s) found in the membrane is open to question. Purity of the membrane fractions are determined by a number of chemical and enzymatic assays.(6) The enzymes, Mg$^{2+}$ activated ATPase, 5' nucleotidase and alkaline p-nitrophenyl phosphatase are all used as membrane
markers(6,7), while glucose-6-phosphatase and cytochrome C Reductase are common microsomal markers used to detect levels of contamination in the plasma membrane fraction.(6-8)

Membrane isolation procedures for this investigation were taken from Scarpulla.(9) This isolation procedure yields a membrane preparation at least 95% pure. Chemical and enzymatic assays, in addition to electron microscopy, were used to determine the purity of the membrane fraction. From the results obtained by Scarpulla, the membrane fraction would require a microsomal contamination of 15-20% in order to account for the RNA component. A comparison of electron micrographs of "pure" membrane fractions and plasma membrane fractions deliberately contaminated with 5, 10, 15, and 20% microsomal material were studied.(9) The results of these experiments illustrated that the "pure" membrane fraction was clearly distinguishable from the plasma membrane sample contaminated with 5% microsomal material. This experiment clearly indicates the plasma membrane procedure used in this study is at least 95% pure. Since this membrane isolation procedure has repeatedly yielded such high purity, this investigation did not concern itself with repeating procedures used to determine membrane purity. Confidence was held that Scarpulla's isolation procedure would yield membrane fractions which were at least 95% pure. Characterization of this RNA component was the major concern of this investigation.

Separations of isoaccepting transfer RNA molecules:
have been reported.\(10-12\) The separations involve column chromatography using a variety of resins including: DEAE-Cellulose(13), DEAE-Sephadex(14), Hydroxylapatite(15), Methylated-Albumin-Kieselguhr(MAK) (16), Reverse Phase Chromatography(RPC) (17), and Benzoylated DEAE-Cellulose (BD-Cellulose) (18).

MAK, RPC, and BD-Cellulose are the three most commonly used column resins. RPC columns require the use of pressurized (30-50psi) columns(19), and give excellent resolution of the isoaccepting species.

Recent advances in the resolution of isoaccepting tRNAs using BD-Cellulose chromatography has prompted many investigators to use this material. The principle of separation involves not only the ionic interactions of the polynucleotide with the resin but also nonionic interactions. Transfer RNAs have many modified bases in their molecular structure, e.g. pseudouridine, thiouridine, and methylinosine. These modified bases interact with the benzoyl groups of the BD-Cellulose resin to bind more strongly than with non-benzoylated cellulose. Benzoylated DEAE-Cellulose columns have been used for separation of isoaccepting tRNAs in a variety of systems including transformed mouse fibroblasts(20), and aerobic and anaerobic cultures of a facultative photoheterotroph.(21) The excellent resolution and reproducability of the chromatographic profiles have led investigators to use Benzoylated DEAE-Cellulose as a column resin.

The RNA component associated with plasma membranes
may be a contaminate of the plasma membrane preparation. An approach to resolving this question is to chromographically characterize the tRNA component by comparing the iso-accepting species of the RNA isolated from the plasma membrane to that of the cytoplasmic tRNA. If a difference can be detected between isoaccepting species, this would give strong support that this RNA component, is in fact, an integral part of plasma membranes.
MATERIALS AND METHODS

Preparation of Plasma Membranes

Plasma membranes were isolated by a modification of the method of Ray.(6) All procedures were carried out at 0-5°C. Homogenizing buffer consisted of 0.5mM CaCl$_2$ and 1.0mM NaHCO$_3$ (pH 7.5). Eight grams of fresh rat liver were minced with scissors and homogenized in 100ml buffer using 25 strokes in large Dounce Homogenizer with a loose pistle (Blaesig Glass Co., Rochester N.Y.).

The homogenate was diluted to 800ml with buffer and left to stand 5 minutes with occasional stirring. The homogenate was filtered through four layers of cheese cloth and centrifuged at 1600xg for 30 minutes (GSA rotor of Sorvall RC2-B refrigerated centrifuge).

The supernatant (I) was saved for isolation of a microsomal fraction. The resulting pellet (I) was suspended in 100ml buffer, gently homogenized, diluted to 400ml, and centrifuged at 1600xg for 15 minutes. The resulting supernatant (II) was discarded and the resulting pellet (II) was suspended in 200ml buffer and centrifuged as above. The final pellet (III) was suspended in buffer (approx. 9.5ml).

The suspension containing pellet (III) was mixed with 20.5ml of 70% sucrose (w/w) in homogenizing buffer to a final concentration of 48% sucrose. Five ml of this suspension were placed in each of 6 Spinco rotor SW 25.1
centrifuge tubes. In each tube was layered 8ml 45% sucrose (w/w), 10ml 41% sucrose (w/w) and finally 37% sucrose (w/w). Tubes were centrifuged for one hour at 64,000xg in a Beckman L3-50 Ultracentrifuge. The band at the interface between 37% and 41% sucrose was collected with a Pasteur pipette. The six membrane bands resulting from this centrifugation were combined (Membrane Fraction #1). Membrane Fraction #1 was mixed with equal volume of buffer and centrifuged at 12,100xg for 10 minutes in Sorvall SS-34 rotor. The resulting pellet was suspended in volume of 3ml buffer. This membrane suspension was mixed with approx. 6.5ml 70% sucrose to a final concentration of 48% sucrose and divided among 2 Spinco SW 25.1 centrifuge tubes. Discontinuous gradients were again prepared, centrifuged and recollected as above.

The second washed plasma membrane fraction (Fraction I2) was divided and layered on two continuous sucrose gradients of 25-50% sucrose (w/w) in Spinco SW 25.1 tubes. The suspension was centrifuged at 64,000xg for two hours. The plasma membranes banded at a density of approx. 1.16g ml⁻¹, were collected, washed and suspended in 2ml of homogenizing buffer. This membrane fraction was labeled 1-G and was used as the source of purified membranes.

Isolation of Microsomes

A microsomal fraction was isolated from the supernate of pellet (1) by centrifugation at 10,800xg for 10 minutes...
in Sorvall SS-34 rotor. The resulting supernate was then centrifuged at 134,800xg for one hour in Beckman L3-50 Ultracentrifuge Ti 50 rotor. The resulting pellet was washed by recentrifugation and suspended in buffer.

**Preparation of Cytoplasmic RNA**

Ten grams of fresh rat liver was mixed with five volumes of 0.1M Tris-Cl (pH 7.5) buffer. This solution was homogenized for one minute with a Sorvall Omni-Mixer. The homogenate was centrifuged for 10 minutes at 27,000xg in Sorvall SS-34 rotor. The aqueous supernatant was mixed with equal volume of water saturated phenol and stirred for five minutes at room temperature. The solution was then centrifuged for 10 minutes at 12,100xg in Sorvall SS-34 rotor. The top aqueous layer was mixed with one tenth volume of 20% potassium acetate pH 5.4 and 2.5 volumes of ethanol. The RNA was collected by centrifugation for five minutes at 3,000xg in Sorvall SS-34 rotor. The RNA was resuspended in 0.1M Tris buffer (pH 7.5).

A DEAE-Cellulose column (17.8 x 1.3cm) was equilibrated with 0.1M NaCl in 0.1M Tris-Cl (pH 7.5). The RNA solution was applied on the column and washed with the above buffer until the absorbance at 260nm was less than 0.10. The RNA was then eluted with 1.5M NaCl in 0.1M Tris-Cl (pH 7.5). Five ml fractions were collected. Fractions with absorbance greater than 0.5 at 260nm were combined and mixed with 2.5
volumes ethanol and the RNA was allowed to precipitate as above.

The RNA precipitate was centrifuged for five minutes at 12,100xg in Sorvall SS-34 rotor and suspended in one ml of distilled water.

Isolation of RNA

RNA was isolated from cellular fractions by sodium dodecyl sulfate (SDS)-phenol extraction. Fractions were mixed with one-tenth volume of 10% SDS and gently homogenized with a pipette. One-half volume of water saturated phenol was added and solution was shaken vigorously. The suspension was centrifuged for 10 minutes at 12,100xg in Sorvall SS-34 rotor. The supernatant was saved and phenol layer was re-extracted with 0.5ml of homogenizing buffer and centrifuged as above. The supernatants were combined and one-tenth volume of 20% sodium acetate (pH 5.4) and 2.5 volumes ethanol were added. The RNA was allowed to precipitate overnight at 0°C. The precipitate was collected by centrifugation for 15 minutes at 27,000xg in Sorvall SS-34 rotor and dissolved in 0.5ml distilled water.

All RNA samples were discharged (de-esterified) of amino acids by incubation in 1.0M Tris-C1 (pH 9.0) at 37°C for 90 minutes. (22) The RNAs were recovered by ethanol precipitation as above and redissolved in distilled water.
Chemical Determinations

Protein was determined by the method of Lowry et al (23) using bovine serum albumin as standard. RNA was determined by the Dische Orcinol technique (24).

Preparation of Aminoacyl Synthetases and Aminoacylation of Transfer RNA

Ten grams of fresh rat liver were homogenized in 50ml buffer "A" [10mM Tris-Cl pH 7.5, 1.0mM MgCl₂, 20mM Beta-Mercaptoethanol, 10mM NH₄Cl and 10% glycerol (v/v)]. The homogenate was centrifuged for five minutes at 27,000xg in Sorvall SS-34 rotor. The supernatant was then chromatographed on a DEAE-Cellulose column (1 x 3cm) previously equilibrated with buffer "A". The column was washed with buffer "A" until absorbance at 280nm was less than 0.1. Protein fractions were then eluted with buffer A' [10mM Tris-Cl pH 7.5, 1.0mM MgCl₂, 20mM Beta-Mercaptoethanol, .25M NH₄Cl and 10% glycerol (v/v)]. Fractions with absorbance greater than 1.0 were combined, concentrated by dialysis against 20% Carbowax in buffer "A", and then dialyzed against buffer "A". The final solution was adjusted to 50% glycerol (v/v) and stored at -20°C.

RNA was aminoacylated in a .1ml reaction mixture containing 10um Na Cacodylate (pH 7.2), .2um ATP, 1.0um potassium chloride, 1.0um magnesium acetate, 1.0uc ³H-labeled amino acid, 2-15ug RNA and 10ul synthetase enzyme (approx. 55ug protein). The mixture was incubated at 37°C and 20ul aliquoits were
removed at 0, 5, 10, 20 minutes, placed on Whatmann 3MM filter discs and placed in cold 5% Trichloroacetic Acid (TCA). The discs were washed three times in cold 5% TCA, once in ether–ethanol mixture (1:1) and finally in ether. Each wash lasted for 15 minutes. The dried discs were counted in Omniflour (New England Nuclear)–Toluene scintillation cocktail mixture (4 grams of Omniflour to one liter of toluene). Reaction mixtures were counted in a Packard Tri Carb Liquid Scintillation Spectrometer. Counting efficiency for tritium was 9%, for carbon-14 64%.

**Large Scale Aminoacylation of Cytoplasmic and Membrane tRNA**

$^{14}$C-labeled amino acids were used for charging of cytoplasmic tRNA while $^3$H-labeled amino acids were used for membrane tRNA.

Cytoplasmic tRNA was aminoacylated in a .5ml reaction mixture containing 50uM Na Cacodylate (pH 7.2), 1uM ATP, 5uM potassium chloride, 5uM magnesium acetate, .75uC 14C-labeled amino acid, 150–200ug RNA and 75ul synthetase enzyme (approx. 400ug protein).

Reaction mixtures were incubated for 10 minutes at 37°C and immediately transferred to ice with the addition of 500ul 1M sodium acetate (pH 5.4), 50ul glacial acetic acid, and 600ul phenol saturated with .1M sodium acetate (pH 5.4). The solution was then shaken thoroughly. At this point the membrane reaction was aided by the addition
of 75ul (375ug) of cytoplasmic carrier RNA. The reaction mixtures were centrifuged for five minutes at 4,300xg in Sorvall SS-34 rotor. The aqueous supernatant was saved and the phenol layer was re-extracted with 500ul of .1M sodium acetate (pH 5.4) and recentrifuged as above. The supernatants were combined with the addition of 2.5 volumes of ethanol. The RNA was allowed to precipitate overnight at 0°C. The RNA precipitate was centrifuged for five minutes at 12,100xg and the resulting pellet was redissolved in 500ul .4M sodium chloride buffer (.01M MgCl₂ and .05M sodium acetate pH 5.0).

The RNA solutions were either chromatographed separately on Benzoylated Diethylaminoethyl Cellulose columns or mixed together and chromatographed for comparative profiles.

Benzoylated Diethylaminoethyl Cellulose Chromatography of Cytoplasmic and Membrane Aminoacyl Transfer RNA

Benzoylated Diethylaminoethyl Cellulose Chromatography (BD-Cellulose Chromatography) profiles of isoaccepting tRNA species were performed by a modification of the method of Taylor.(25) BD-Cellulose was obtained from Schwarz/Mann Co. All columns (40.5 x .6cm) were previously equilibrated with .4M sodium chloride buffer (.01M MgCl₂ and .05M sodium acetate pH 5.0), and were run at 4°C. Solutions of tRNA in .4M NaCl buffer were placed on the column, washed with
200ul of .4M NaCl buffer and the aminoacylated tRNA was eluted with a linear gradient (60ml of .4M NaCl buffer and 60ml of 1.2M NaCl buffer). The flow rate was 14ml/hr. 700ul fractions were collected. At the completion of the gradient an additional 40ml of 1.2M NaCl buffer and then 40ml 1.2M NaCl buffer containing 10% ethanol was washed through the column. A 500ul aliquot from each fraction was placed in a Triton X-100 (Sigma Chemicals) Toluene scintillation cocktail (100ml Triton X-100, 275ml toluene and 2.2g Omniflor).(26) 3.5ml of cocktail solution was placed in each counting vial. Vials were then counted in a Packard Tri-Carb Liquid Scintillation Spectrometer. For a typical column profile 45-60,000 counts per minute of aminoacylated cytoplasmic tRNA (carbon-14 labeled) and 45-80,000 counts per minute of aminoacylated membrane tRNA (tritium labeled) were applied to the column.
RESULTS

Properties of Plasma Membranes

The properties of rat liver plasma membranes with respect to protein and RNA content are summarized in Table I. The range observed is due in part to biological variability and statistical experimental error from the ten preparations used. These results are comparable to that of previous reports.(9)

Aminoacylation of RNA Extracted from Membranes

RNA extracted from cytoplasmic and plasma membrane fractions was assayed for transfer RNA activity and typical results are shown for proline and tryptophan in Figures 1 & 2 respectively. Since the assay conditions for charging of amino acids vary, the results obtained may not be optimal. To aid in detecting the aminoacyl tRNAs, additional 50mM ATP was added to reaction mixtures. A typical graph of such an assay involving proline is illustrated in Figure 3. This additional 50mM ATP aided in the detection of small amounts of tRNA from the plasma membrane fraction. Table II gives a typical set of raw data and Table III gives a summary of the results of aminoacylation experiments with four amino acids. In addition to confirming acceptor activity for arginine and lysine from Scarpulla's data(9), acceptor
TABLE I

PROTEIN AND RNA CONTENT OF PLASMA MEMBRANES

<table>
<thead>
<tr>
<th>PLASMA MEMBRANE</th>
<th>AVERAGE*</th>
<th>RANGE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg Protein/g liver</td>
<td>1.14 ± .185</td>
<td>.63-2.7</td>
</tr>
<tr>
<td>ug RNA/mg Protein</td>
<td>12.3 ± 1.03</td>
<td>8.1-19.8</td>
</tr>
</tbody>
</table>

* Based on 10 Preparations
Figure 1

AMINOACYLATION ASSAY OF RNA FROM MEMBRANE FRACTION AND LOW MOLECULAR WEIGHT CYTOPLASMIC RNA

Specific activity of $^3$H proline was 37.3 Ci/m mole. Control with no RNA added (x—x). Incorporation of $^3$H proline by membrane fraction RNA 24µg (O—O) and 96µg (O—O) and by low molecular weight cytoplasmic RNA 12.5µg (□—□) and 25µg (□—□). Assay conditions are described in the text.
Figure 2

AMINOACYLATION ASSAY OF RNA FROM MEMBRANE FRACTION AND LOW MOLECULAR WEIGHT CYTOPLASMIC RNA

Specific activity of $^3$H tryptophan was 2.1 Ci/m mole. Control with no RNA added (x--x). Incorporation of $^3$H tryptophan by membrane fraction RNA 23ug (O-O) and 43ug (O-O) and by low molecular weight cytoplasmic RNA 5ug (O-O) and 12.5ug (O-O). Assay conditions are described in the text.
Figure 3

AMINOACYLATION ASSAY OF LOW MOLECULAR WEIGHT CYTOPLASMIC RNA WITH ADDITIONAL 20mM ATP ADDED TO REACTION MIXTURE

Specific activity of $^3$H proline was 37.3 Ci/mmole. Control with no RNA added (x-x). Incorporation of $^3$H proline by low molecular weight cytoplasmic RNA under normal assay conditions, 12.5ug (O-O) and with additional 20ul of 50mM ATP for final concentration of 0.4uM in reaction mixture, 12.5ug (0-0). Assay conditions are described in the text.
### TABLE II

AMINO ACID ACCEPTOR ACTIVITY OF RNA ISOLATED FROM CELL FRACTIONS

Values in counts per minute amino acid incorporation into RNA

#### PROLINE

<table>
<thead>
<tr>
<th>TIME (min.)</th>
<th>0 RNA</th>
<th>23ug MEMB. RNA</th>
<th>12.5ug CYTO. RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>64</td>
<td>97</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>169</td>
<td>515</td>
</tr>
<tr>
<td>10</td>
<td>66</td>
<td>193</td>
<td>863</td>
</tr>
<tr>
<td>20</td>
<td>87</td>
<td>215</td>
<td>978</td>
</tr>
</tbody>
</table>

#### TRYPTOPHAN

<table>
<thead>
<tr>
<th>TIME (min.)</th>
<th>0 RNA</th>
<th>43ug MEMB. RNA</th>
<th>12.5ug CYTO. RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>520</td>
<td>843</td>
<td>587</td>
</tr>
<tr>
<td>5</td>
<td>684</td>
<td>1216</td>
<td>2485</td>
</tr>
<tr>
<td>10</td>
<td>599</td>
<td>1029</td>
<td>2454</td>
</tr>
<tr>
<td>20</td>
<td>569</td>
<td>1299</td>
<td>2249</td>
</tr>
</tbody>
</table>

#### ARGinine

<table>
<thead>
<tr>
<th>TIME (min.)</th>
<th>0 RNA</th>
<th>3.2ug MEMB. RNA</th>
<th>3.6ug CYTO. RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>98</td>
<td>799</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>211</td>
<td>3032</td>
</tr>
<tr>
<td>10</td>
<td>28</td>
<td>238</td>
<td>4424</td>
</tr>
<tr>
<td>20</td>
<td>28</td>
<td>277</td>
<td>3581</td>
</tr>
</tbody>
</table>

#### LYSINE

<table>
<thead>
<tr>
<th>TIME (min.)</th>
<th>0 RNA</th>
<th>3.2ug MEMB. RNA</th>
<th>3.6ug CYTO. RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16</td>
<td>25</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>64</td>
<td>204</td>
<td>1687</td>
</tr>
<tr>
<td>10</td>
<td>89</td>
<td>231</td>
<td>2021</td>
</tr>
<tr>
<td>20</td>
<td>132</td>
<td>294</td>
<td>2212</td>
</tr>
</tbody>
</table>
**TABLE III**

**AMINO ACID ACCEPTOR ACTIVITY OF RNA ISOLATED FROM CELL FRACTIONS**

Values in pmoles amino acid acylated per ug RNA

<table>
<thead>
<tr>
<th></th>
<th>MEMBRANES</th>
<th>CYTOPLASM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARGinine</td>
<td>0.07</td>
<td>1.50</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.02</td>
<td>0.76</td>
</tr>
<tr>
<td>Proline</td>
<td>0.04</td>
<td>0.128</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.18</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Assay conditions as described in text. Specific activities of amino acids were as follows: arginine, 27.3 Ci/mmmole, lysine 55 Ci/mmmole, proline, 37.3 Ci/mmmole, tryptophan, 2.1 Ci/mmmole.
activity for proline and tryptophan were detected. Table III represents aminoacylation experiments performed with two separate plasma membrane preparations.

**BD-Cellulose Chromatography Profiles of Arginine and Lysine Isoaccepting tRNAs**

Chromatographic profiles of arginine and lysine isoaccepting species from cytoplasmic and membrane tRNA are illustrated in Figures 4 & 5 respectively. Cytoplasmic RNA was labeled with $^{14}$C amino acids while plasma membrane RNA was labeled with $^3$H amino acids. The addition of ethanol at the end of the NaCl gradient was to remove any species still bound to the BD-Cellulose resin. As can be seen from the elution profiles, there are no major differences between isoaccepting species of cytoplasmic and membrane tRNA for arginine or lysine. All peaks including the ethanol peak are similar in nature. The major peak for arginine eluted at a NaCl concentration of 0.6M, while lysine eluted at 0.7M. The first peak in each profile, run off peak, (data not shown) was due to the free amino acids eluting through the column. From the elution profiles obtained it can be stated that neither arginine nor lysine isoaccepting species show any major difference between cytoplasmic and membrane tRNA. These chromatographic profiles are typical for two separate membrane preparations for each amino acid used.
BD-CELLULOSE CHROMATOGRAPHY OF ARGinine TRANSFER RNA

Figure 4

Counts per 0.5 ml per 5 min.

Fraction Number

NaCl Concentration (M/L)

14C CYTOPLASMIC ARG-tRNA

3H MEMBRANE ARG-tRNA

ETHANOL ADDED
BD-CELLULOSE CHROMATOGRAPHY OF LYSINE TRANSFER RNA

Figure 5

COUNTS PER 0.5mL PER 5 MIN.

FRACTION NUMBER

NaCl CONCENTRATION (M/L)

3H MEMBRANE LYS-tRNA

14C CYTOPLASMIC LYS-tRNA

ETHANOL ADDED
DISCUSSION

The existence of RNA associated with plasma membranes is a widely observed phenomenon. This investigation has clearly shown that an RNA component is associated with the plasma membrane. It has also characterized arginine and lysine tRNA via their isoaccepting species. Membrane purity is the single most determining factor in proving this RNA component is not due to contamination. The procedures used in this study yielded a membrane fraction which was at least 95% pure. The proof necessary to answer this question as to whether RNA does exist in membranes will rest on the membrane fraction being of the highest purity.

Transfer RNA is the easiest type of RNA to detect. An aminoacylation assay is all that is needed to determine its presence. This ease of detection made it possible to investigate the existence of tRNA in the membrane. It is clear from this data and previous data (Scarpulla '74) that there are at least five different tRNA species associated (present in?) the membrane. Arginine, lysine, phenylalanine, proline, and tryptophan tRNA acceptors are all present in the membrane fraction. This leaves fifteen amino acids left to check for acceptor activity.

Because of the low concentrations of cytoplasmic and membrane tRNA used, it was difficult to obtain a linear aminoacylation curve. Although higher counts were achieved with the addition of RNA, linearity was not always obtained.
The reason why radioactive counts decreased with an increase in RNA, as indicated by membrane RNA with proline, is not fully understood. Experimental error could have been a factor or some degradation of the RNA sample during the assay could have caused the observed results. Despite the drop in counts, it is clear from the pmoles/ug RNA ratio that proline tRNA is present in the membrane fraction.

The isoaccepting species characteristic of transfer RNA were chromatographed on Benzolated DEAE-Cellulose columns. The cytoplasmic tRNA was labeled with $^{14}$C amino acids while the membrane tRNA was labeled with $^3$H amino acids. As can be seen from the graphs, both cytoplasmic and membrane tRNAs demonstrated similar chromatographic profiles. This data does not help to solve this problem of determining whether or not RNA is an integral part of the plasma membrane. The only conclusion that can be drawn from these results is that the isoaccepting tRNA species for arginine and lysine profile in a similar manner. This is not to say that the plasma membrane tRNA is a contaminant from the cytoplasm. It shows that for two of the five amino acids detected from the cytoplasm and membrane fractions, the isoaccepting species are similar.

Another aspect of the data that should be discussed are the ratios of the amino acids detected in the cytoplasmic and plasma membrane fractions. A previous report (27) compared the amino acid-acceptor activities of viral and cellular tRNAs. If the data from Table III are converted
to ratios with arginine set as one, the results can be seen in Table IV. This information will aid in determining whether this RNA component is a contamination from the cytoplasm. Assuming the RNA detected in the plasma membrane is a contaminant, the ratios of these amino acids should be the same for both fractions. Clearly, the results in Table IV show a difference between the cytoplasmic and plasma membrane fractions. The numbers obtained from these ratios are directly related to the aminoacylation assay results. As was previously stated, the results from those experiments were not linear, therefore the validity of the ratio results can be questioned. But the numbers in Table IV definitely show a difference between the cytoplasmic and plasma membrane fractions and this would tend to support the possibility that RNA is, in fact, associated with the plasma membrane.

Problems were encountered in establishing column conditions for cytoplasmic isoaccepting species. Taylor (26) was able to show four isoaccepting species for lysine tRNA. This investigation was able to resolve two species with shoulders on the peaks possibly representing other species. The arginine profile from Portugal et al (20) was reproducible with much more success. The one peak shown by Portugal was also reproduced by this investigation. The column conditions were exactly the same for both cytoplasmic and membrane samples. Therefore, if there was a difference between the cytoplasmic and membrane sample it would have been seen by the profiles.
TABLE IV

RATIOS OF AMINO ACIDS DETECTED FOR PLASMA MEMBRANE AND CYTOPLASMIC FRACTIONS

<table>
<thead>
<tr>
<th></th>
<th>PLASMA MEMBRANE</th>
<th>CYTOPLASM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>3.5</td>
<td>1.97</td>
</tr>
<tr>
<td>Lysine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>1.75</td>
<td>11.7</td>
</tr>
<tr>
<td>Proline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>.39</td>
<td>.71</td>
</tr>
<tr>
<td>Tryptophan</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data obtained from Table III. Ratios expressed as pmoles amino acid acylated per ug RNA.
Although the isoaccepting species for arginine and lysine profiled similarly no conclusion can be made as to whether or not the RNA was due to contamination.

Although the experiments performed during this investigation did not check the purity of the membrane fraction, the values obtained for protein and RNA content are comparable to that of Scarpulla's. Even the aminoacylation of lysine with membrane RNA is identical to previous data. These results lead one to believe that the membrane preparation used in this investigation was at least 95% pure. Because the membrane preparation used is not absolutely pure, contamination cannot be entirely ruled out. It is still a real possibility that this RNA being detected is caused by cytoplasmic contamination.

The finding of an RNA related process on the membrane would give strong support for RNA being an integral part of the plasma membrane. Consideration must be given to a possible function of RNA in the membrane. The transfer RNA detected could serve as a transport molecule to allow amino acids to move across the membrane. The possibility of a protein synthesizing system in the plasma membrane might be the answer to this question. Incorporation of amino acids into polypeptides by a surface membrane preparation has been reported. This system is ribosomal dependent and the observed results might be due to microsomal contamination.

Protein synthesis devoid of ribosomes might also be
a function of the tRNA component.(30) An outside stimulus such as a hormone on its specific binding site might activate the synthesizing process.

Transfer RNA may not be the only RNA species to be found associated with membranes. If messenger or ribosomal RNA can also be associated with the plasma membrane it may aid in determining what the biological significance of RNA in the membrane might be.

The cell is one of the most efficient "machines" known. If RNA is in fact shown to be an integral part of the plasma membrane there must be a biological function associated with it.

The results presented here clearly indicate an association of RNA, and specifically tRNA, with plasma membranes. The biological significance of this preliminary finding is still unknown. More work is needed to obtain higher purity with respect to membrane fractions. The question as to whether this RNA is a real component of membranes will rest on further refinement of membrane isolation and finding a biological function for this component.
5. Juliano, R., Ciszkowski, J., Waite, D., and Mayhew, E.,
7. Coleman, R., Michell, R.H., Finean, J.B., and Hawthorne, J.N.,
8. Mackler, B., Meth. in Enzymol. 10: 551 (1967)
12. Maxwell, L.H., Wimmer, E., and Tener, G.M., Biochemistry 7:
   2629-2634 (1968)
13. Cherayil, J.D., and Boch, R.M., Biochemistry 4: 1174 (1965)
15. Muench, H.K., Proced. in Nucleic Acid Res. 2: 515 (1971)
16. Sueoka, T., Proced. in Nucleic Acid Res. 2: 608 (1971)
18. Gillam, I., Millward, S., Blew, D., Tigerstrom, M., Wimmer, E.,
    and Tener, G.M., Biochemistry 6: 3043-3056 (1967)
    Biophys. Acta 228: 770-774 (1971)