Characterization of RNA Associated with Rat Liver Plasma Membranes

John J. DeBellis

The College at Brockport

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CHARACTERIZATION OF RNA ASSOCIATED WITH RAT LIVER PLASMA MEMBRANES

BY

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State University of New York
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in partial fulfillment of
the requirements for the
degree of Master of Science

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THESIS DEFENSE

CHARACTERIZATION OF RNA ASSOCIATED WITH
RAT LIVER PLASMA MEMBRANES

BY

JOHN J. DEBELLIS

APPROVED

NOT APPROVED

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Thomas P. Bonica 6/14/77
Committee Member Date

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Theodore J. Starr
Chairman, Dept. Biological Sciences
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ACKNOWLEDGEMENTS

I wish to thank Dr. Larry K. Kline for his excellent guidance and supervision during this research project and his helpful advice during the past two years of my graduate studies. I also wish to acknowledge my mother, for her encouragement during this research project.
ABSTRACT

RNA was extracted from purified rat liver plasma membranes. The RNA was characterized in terms of molecular weight distribution (electrophoresis) and base composition. Plasma membrane RNA was shown to have a major 28S species, several minor 15-22S species and another minor 4S species. There was no difference between plasma membrane RNA and ribosomal RNA in terms of base composition. The intact plasma membrane was incubated in the presence of RNAse as well as varying concentrations of NaCl. These results demonstrate that the RNA associated with the plasma membrane is partially digested with RNAse, while 0.15 M NaCl seems to have little effect on the plasma membrane RNA content. This may indicate that the plasma membrane RNA is protruding from the plasma membrane but is attached to the plasma membrane in some manner. Incubation of intact plasma membranes with 0.30 M NaCl (final concentration) removes 63.2% of the RNA associated with the plasma membrane. The species of RNA released is unknown.
INTRODUCTION

Membranes are found in all types of cells. In eucaryotic cells, membranes are associated with cellular organelles and the cell itself. The nucleus, lysosome, golgi apparatus, mitochondria and chloroplast are structured by internal membranes, while the cell is encompassed by the plasma membrane. Internal membranes function to separate the organelle's contents from the cytoplasmic matrix, whereas the plasma membrane separates the cell from the external environment.

The plasma membrane, in addition to protection of the cell, has many other functions associated with it. Among some are: intercellular adhesion, selective permeability to ions, hormone interactions, cellular recognition and mobility of the cell.

The structure of the plasma membrane has been studied extensively by the use of such techniques as: x-ray scattering, electron microscopy and spin labeling of fatty acids. Over the years, many models of the plasma membrane have been postulated, ranging from the Overton model of a continuous layer of lipid material to the Davson and Danielli model of a phospholipid bilayer sandwiched by two layers of proteins. Recent studies have demonstrated that a portion of the protein molecules may actually transcend the membrane and may be partly in the form of glycoproteins. These proteins have a certain fluidity within the lipid bilayer and seem to be independent of the lipid bilayer.

Plasma membranes have been demonstrated to be composed
of phospholipid, protein, carbohydrate, cholesterol and fatty acids. In addition, ribonucleic acid (RNA) associated with the plasma membrane, has been detected by nearly every investigator who has isolated plasma membranes. However, the question remains: Is this RNA a contaminant from the cell, adhering to the plasma membrane during isolation or is it indeed associated with the plasma membrane?

It has been demonstrated that RNA is associated with the plasma membrane in the form of a liporibonucleoprotein complex within the plasma membrane. Others claim that the RNA is associated on the periphery of the plasma membrane.

To date, no biological function has been assigned to this plasma membrane RNA, if it is indeed a genuine component of the plasma membrane. Many functions for the plasma membrane RNA have been postulated, ranging from an integral structural component to receptor sites for various molecules and organelles (i.e. hormones and ribosomes).

Previous studies have shown that the plasma membrane associated RNA is 10-20% transfer RNA. The purpose of this investigation is to characterize other species of RNA that may be associated with the plasma membrane. That is, to compare the base composition and species molecular weight of plasma membrane RNA and other cellular RNAs.
RNA has been detected in practically all membranes of the animal cell (i.e. mitochondrial, endoplasmic reticulum, nuclear and plasma membranes). This investigation concerns RNA associated with the plasma membrane. The question of whether it is actually associated with the plasma membrane or is an impurity, is an unresolved problem. The procedures used in this investigation yield a plasma membrane fraction that is at least 95% pure.

There are three major classifications of RNA species located within the cell, ribosomal RNA (28S, 18S and 5S), messenger RNA (ranging from 6S to 20S) and transfer RNA (4S). It has been previously demonstrated that plasma membrane RNA contains 10-20% transfer RNA; the nature of the remaining 80-90% of the RNA is still under investigation.

The purpose of this investigation is to characterize plasma membrane RNA by:
1) Electrophoretically determining the molecular weights of plasma membrane RNA species,
2) Determining the base composition of the plasma membrane RNA.

The location of the RNA associated with the plasma membrane is of interest to many investigators. Whether the RNA is associated with the lipid bilayer or the proteins remains to be established. It would, therefore, be of interest to:
3) Determine the manner of physical association of the RNA with the plasma membrane, by removal of peripheral proteins and the action of RNase.
These studies may lead to a possible assignment of the biological function of plasma membrane RNA.
The components and structure of the plasma membrane have been studied extensively. It is now established that the components of the plasma membrane consist of a phospholipid bilayer, proteins and carbohydrates in the form of glycolipids and glycoproteins (1). The structure of the plasma membrane is such that it is thermodynamically stable due to the interactions of the hydrophobic (hydrocarbons) and hydrophilic (phosphate group) components of the phospholipid bilayer (2,3). The proteins associated with the plasma membrane are of two classes, integral and peripheral. The integral proteins extend across the lipid bilayer and are thought to be amphipathic, with the hydrophobic interior region of the protein embedded in the lipid bilayer. The peripheral proteins are associated with the integral proteins, by either electrostatic or hydrophobic binding (2,3). In addition to these components, a ribonucleic acid (RNA) component has been detected by a number of investigators (4-16).

The hepatocyte plasma membrane is of particular interest, as it is not a uniform structure in vivo. Modifications, such as bile canaliculi, desmosomes, tight junctions and microvilli are common among hepatocyte plasma membranes (17).

Several methods for the isolation of plasma membranes have been described. Initial procedures called for the disruption of the cells by homogenization in a sodium bicarbonate buffer (pH 7.5), followed by low speed differential centrifugation and ultimately floatation of the membrane.
material in a sucrose gradient (18). This method results in plasma membrane preparations that are devoid of contaminants from other cellular components (see below). Modifications, such as the use of isotonic sucrose buffers were added to preserve the integrity of other cellular components and reduce the chance of contamination (19). Another method for the isolation of plasma membranes is by the use of an aqueous two phase polymer system (dextran-polyethylene-glycol system) (20,21). This procedure does not utilize the floatation of membrane material on sucrose gradients. The plasma membrane fraction forms at the interface between the two polymers and purity of these plasma membranes are comparable to plasma membranes obtained from floatation methods (20).

The "purity" of plasma membranes may be determined by the use of electron microscopy (4,22) and a number of chemical and enzymatic assays (4,22,23). Enzymes located on the plasma membrane include: 5'-nucleotidase, acid and alkaline phosphatase, adenyl cyclase, Mg$^{2+}$-activated ATPase and p-nitrophenyl phosphatase. Plasma membrane contamination may be determined by the enzymes glucose-6-phosphatase and NADH$_2$-cytochrome-c reductase, which are associated with the microsomes (22) and succinate dehydrogenase which is associated with the mitochondria (24).

The isolation procedure that was used for these experiments were taken from the procedure of Scarpulla (12). This plasma membrane preparation is at least 95% pure as determined by electron microscopy and enzymatic assays. The plasma membrane
fraction was deliberately contaminated with 5, 10, 15 and 20% microsomal material, and it was determined that 5% microsomal contamination results in an additional six micrograms of RNA in the plasma membrane fraction. It was also demonstrated by electron microscopy, that "pure plasma membranes" are clearly distinguishable from plasma membranes contaminated with 5% microsomal material. This clearly demonstrates that Scarpulla's procedure yields plasma membranes which are at least 95% pure.

Several investigators have suggested that the RNA from the plasma membrane is an artifact due to isolation procedures (i.e. that the RNA is attracted to the plasma membrane by electrostatic interactions or other mechanisms during the plasma membrane isolation). Other investigators believe that the RNA is an integral constituent of the plasma membrane in the form of a liporibonucleoprotein complex (5,6). Shapot and Davidova (5,6) have purified plasma membranes that were isolated from rat liver, and solubilized 26-28% of the plasma membrane proteins by extraction with 0.14 M NaCl (this removes peripheral proteins). There was no RNA found in the protein fraction. However, when the lipoprotein fraction was dissolved in 8 M urea (or 0.2% sodium dodecyl sulfate), RNA was detected (this removes integral proteins). It was also demonstrated that the RNA present in the intact plasma membrane was resistant to the action of RNase. Recently, a liporibonucleoprotein complex has been isolated from rat liver plasma membranes. The composition of the complex is: 14% lipid,
22.5% protein and 63.5% RNA (7).

Other investigators believe that the RNA is associated with the peripheral proteins (possibly in addition to a liporibonucleoprotein complex). RNA has been detected in the peripheral regions of human osteogenic sarcoma cells (RPMI #41) and L1210 mouse leukemic cells (8). When these cells are "pushed off" the substrate they are attached to, there remains a substance behind, which can be removed by the addition of RNAse, and is therefore thought to be RNA. However, it is not clear whether this RNA is from the peripheral zone or from cell rupture. It has also been demonstrated that when RPMI #41 cells (intact cells) are treated with RNAse, their electrophoretic mobility is reduced, indicating RNA on the periphery (8). Rieber and Bacalao (9) using Chinese hamster ovary cells have demonstrated that RNA is obtainable after treatment of intact cells with trypsin.

The possibility that plasma membrane RNA is different than cytoplasmic RNA has been studied (10). It was demonstrated, by such techniques ranging from RNA digestion with RNAses to the RNA content (i.e. RNA/protein ratio) of plasma membranes isolated by various methods, that plasma membrane RNA is similar to ribosomal RNA. It has also been demonstrated that lysyl-transfer RNA and arginyl-transfer RNA are similar in both the cytoplasm and plasma membrane (11).

The RNA associated with the plasma membrane may be a contaminant due to the plasma membrane preparation. Comparing the base composition of plasma membrane RNA to other cellular
RNAs and determining the molecular weight of plasma membrane RNA may resolve this question. If, indeed, differences are found this would give strong support that RNA is an integral part of the plasma membrane.
MATERIALS AND METHODS

Preparation of Plasma Membranes

Plasma membranes were isolated by a modification of the method of Ray (4). All procedures were performed at 0-5°C. The homogenizing buffer consisted of 0.5 mM CaCl₂ and 1.0 mM NaHCO₃ (pH 7.5). Eight grams of fresh rat liver were minced with scissors and homogenized with 100 ml buffer, using 25-30 gentle strokes in a large Dounce homogenizer with a loose pestle (Blaessig Glass Co., Rochester, New York).

The homogenate was diluted to 800 ml with homogenizing buffer and left to stand for five minutes with occasional mixing. The diluted homogenate was filtered through four layers of cheese cloth and centrifuged at 1600 x g (3100 rev min⁻¹) for 30 minutes (GSA rotor of a Sorvall RC-5 refrigerated centrifuge).

The supernate (I) was saved for isolation of the microsomal fraction. Pellet I was resuspended in 100 ml buffer, by gentle homogenization, diluted to 400 ml and centrifuged at 1600 x g for 15 minutes. The resulting supernate (II) was discarded and pellet II was resuspended in 100 ml buffer, by gentle homogenization, diluted to 200 ml and centrifuged as above. The supernate (III) was discarded and pellet III was resuspended in 12 ml buffer.

Pellet III suspension was mixed with 26.2 ml 70% sucrose (w/w) in homogenizing buffer to yield a 48% sucrose solution, which was distributed among six Spinco SW 25.1 centrifuge
tubes. Over each was layered 8 ml 45\% sucrose (w/w), 10 ml 41\% sucrose (w/w) and 37\% sucrose (w/w) to the top of the tube. The tubes were centrifuged at 64,700 x g (25,000 rev min\(^{-1}\)) for 1 hour in a Beckman L3-50 ultracentrifuge (SW 25.1 rotor). The plasma membrane obtained at the 37\% and 41\% sucrose interface were collected with a pasteur pipet. The 6 membrane bands were pooled and designated Membrane Fraction #1. Membrane Fraction #1 was mixed with an equal volume of homogenizing buffer and centrifuged at 12,100 x g (10,000 rev min\(^{-1}\)) for 10 minutes in a Sorvall SS-34 rotor. The resulting pellet was resuspended in 6 ml buffer. This membrane suspension was mixed with 13.1 ml 70\% sucrose to yield a 48\% sucrose solution, which was distributed among 3 Spinco SW 25.1 centrifuge tubes. Discontinuous gradients were again prepared, centrifuged and collected as above. The resulting pellet was resuspended in a final volume of 3-4 ml buffer and designated Membrane Fraction 1-G.

Membrane Fraction 1-G has been previously demonstrated to be at least 95\% pure by enzymatic assays and electron microscopy (12).

### Preparation of Microsomes

A microsomal fraction was isolated from supernatant I obtained from the plasma membrane isolation. The supernate (I) was centrifuged at 10,800 (9500 rev min\(^{-1}\)) for 10 minutes in a Sorvall SS-34 rotor. The resulting mitochondrial pellet was discarded and the supernate centrifuged at 134,800 x g
for 1 hour in a Beckman L3-50 ultracentrifuge Ti-50 rotor. The resulting pellet was resuspended by homogenization (5-10 strokes) in buffer and washed by recentrifugation as above. The resulting pellet was resuspended in a final volume of 3 ml buffer.

**Preparation of E. coli Ribosomes**

*E. coli* ribosomes were isolated by a modification of the method of Nirenberg and Matthaei (25). Five grams of *E. coli* were mixed with 10 grams of alumina and ground for 5-10 minutes using a mortar and pestle. To this mixture 10 ml of standard buffer [0.01 M Tris (pH 7.8), 0.01 M magnesium acetate, 0.06 M KCl and 0.006 M 2-mercaptoethanol] was added. The mixture was centrifuged at 20,000 x g (13,000 rev min⁻¹) for 30 minutes in a Sorvall SS-34 rotor. The resulting pellet was discarded and the supernate was centrifuged at 134,800 x g (45,000 rev min⁻¹) for 1 hour in a Beckman L3-50 ultracentrifuge Ti-50 rotor. The resulting pellet was resuspended by homogenization (5-10 strokes) in standard buffer and washed by recentrifugation as above. The resulting pellet was resuspended in a final volume of 1-2 ml standard buffer.

**Isolation of RNA**

RNA was isolated from cellular fractions by sodium dodecyl sulfate (SDS)-phenol extraction. One-tenth volume of SDS was added to the cellular fraction and gently mixed with a pasteur pipet. One-half volume of water saturated phenol was added to
the solution and mixed vigorously. The suspension was centrifuged at 12,100 x g (10,000 rev min\(^{-1}\)) for 10 minutes in a Sorvall SS-34 rotor, to separate the phases. The aqueous phase was removed and the phenol layer was re-extracted with an equal volume of homogenizing buffer and centrifuged as above. To the combined aqueous layers was added 0.1 volume 20% sodium acetate (pH 5.4) and 2.5 volumes 95% ethanol. The RNA was allowed to precipitate overnight at 0°C. The precipitate was collected by centrifugation at 27,000 x g (15,000 rev min\(^{-1}\)) for 15 minutes in a Sorvall SS-34 rotor and resuspended in 0.5 ml distilled water (designated RNA Fraction #1).

RNA Fraction #1 was reprecipitated with ethanol and centrifuged as above. The RNA pellet was resuspended in a final volume of 100-200 ul distilled water.

**Chemical Determinations**

Protein was determined by the method of Lowry et al (26), using Bovine Serum albumin as standard. RNA was determined by the Dische Orcinol technique (27), using E. coli transfer RNA as standard.

**Preparation of Agarose-Polyacrylamide Gels**

Agarose-polyacrylamide gels were prepared according to the method of Dingman and Peacock (28,29). The polyacrylamide gels were strengthened with the addition of 0.5% agarose. Four solutions were used in the preparation of the gels:
(1) 20% acrylamide solution (19% acrylamide, 1% N,N'-methylene-bisacrylamide), (2) 1.6% ammonium persulfate solution, (3) 4% N,N,N,N'-tetramethylethylenediamine (TEMED) (v/v) and (4) 10X electrophoresis buffer [0.89 M Tris, 0.025 M disodium EDTA and 0.89 M Boric acid] (pH 8.3).

A 32 ml 2.5% polyacrylamide gel solution was prepared. To 0.16 g agarose was added 21.8 ml distilled water. This solution was refluxed at 100°C for 15 minutes. The solution was allowed to cool to 37-45°C and 3.2 ml 10X electrophoresis buffer, 2.0 ml TEMED, 4.0 ml 20% acrylamide solution and 1.0 ml 1.6% ammonium persulfate solution were added and mixed. The gel solution was distributed among 10 electrophoresis tubes and topped with water. The gels polymerized in approximately 1 hour.

**Electrophoresis**

Gel electrophoresis was performed according to the method of Dingman and Peacock (28-30). The gels were cut on top to yield a flat surface on which to layer the RNA solution. The gel dimensions were 0.7 x 9.0 cm. The gels were pre-run at 2.5 milliamps per tube in 1X electrophoresis buffer for 30 minutes.

Samples of RNA were diluted at least 1:4 with 40% sucrose in water (w/w). The optimum amount of RNA to layer on a gel is 5-20 μg, with the optimum volume of 15-25 ul.

The RNA solution was placed on the gel and 2.5 milliamps per tube was applied to the gels for 1.25 hours. At the end of the run, the gels were fixed in 1 N acetic acid for 15 minutes.
and stained in 0.2% methylene blue in 0.2 N acetic acid-0.2 N sodium acetate (pH 4.7) for 1 hour at 4°C. The gels were destained by rinsing the gels in successive changes of water at 4°C.

The gels were scanned at 660 nm using a Quick Scan Junior scanner (Helena Laboratories Corp., Beaumont, Texas).

Base Analysis of RNA

Base composition of RNA was performed by the method of Katz and Comb (31). Approximately 40 ug of RNA was hydrolysed in 0.5 ml 0.3 N KOH for 18 hours at 37°C. The solution was neutralized with the addition of 6 N HClO₄ at 0°C and the precipitate was removed by centrifugation. An equal volume of 0.1 N HCl was added to this solution. The sample was applied to a Dowex 50-H⁺ column, 0.9 x 2.0 cm (200-400 mesh, four times cross-linked). The column had been previously washed with 3 N HCl, water until pH 4 and equilibrated with 15 ml 0.05 N HCl.

The quantities of the bases were determined spectrophotometrically as described (31).

Treatment of Intact Plasma Membranes with Pancreatic RNAse and NaCl

In order to gain some insight for the physical properties of the RNA associated with the plasma membrane, the effects of RNAse and varying concentrations of NaCl on the RNA were studied.

Intact plasma membranes (~1 ml, ~5.5 mg) were treated with
either pancreatic RNAse (final concentration of 0.073 ug/ml) or varying concentrations of NaCl (final concentrations of 0.075 M, 0.15 M and 0.30 M). The plasma membranes were incubated at room temperature for 1 hour and centrifuged at 12,800 x g (10,000 rev min⁻¹) for 10 minutes in a Sorvall SS-34 rotor. The membrane pellets were resuspended in a small volume of homogenizing buffer and recentrifuged as above. The supernate from each centrifugation were pooled and protein was determined. The remaining membrane pellet was resuspended in a small volume of homogenizing buffer, protein was determined and RNA was extracted.
RESULTS

Properties of Plasma Membranes

The properties of hepatocyte plasma membranes with respect to protein and RNA content are summarized in Table I-A. These results fall within the range of a plasma membrane preparation that has been shown to be at least 95% pure (12). The results obtained in these experiments are also comparable to the RNA content of plasma membranes reported by investigators who have isolated "purified plasma membranes" (Table I-B).

Electrophoresis

Running conditions for electrophoresis were determined using \textit{E. coli} ribosomal RNA as a standard. It was determined that optimal conditions were obtained with 2.5% polyacrylamide gels (see materials and methods) and applying 2.5 milliamps per tube for 1.25 hours. The optimal amount of RNA to layer on a gel is 5-20 ug, with an optimal volume of 15-25 ul.

RNA extracted from plasma membranes was subjected to electrophoresis, and a typical electrophoretic scan of plasma membrane RNA is shown in Figure 1. Typical electrophoretic scans of plasma membrane RNA with an internal \textit{E. coli} ribosomal RNA marker and \textit{E. coli} ribosomal RNA alone, are shown in Figure 2. These three electrophoretic scans illustrate that plasma membrane RNA consists of a major species heavier than 23S RNA, several minor species in the range of 15-22S RNA and another minor species less than 5S RNA, probably 4S RNA. To elucidate
<table>
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<th>AVERAGE**</th>
<th>RANGE**</th>
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<td>mg protein/g liver</td>
<td>$1.77 \pm 0.22$</td>
<td>0.975-3.10</td>
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<tr>
<td>ug RNA/mg protein</td>
<td>$16.33 \pm 1.27$</td>
<td>10.55-22.57</td>
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Values are given ± standard error

**Based on 10 preparations
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<th>Investigator</th>
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<td>This study</td>
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<td>Emmelot, P. and Bos, C.J. (13)</td>
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<td>Ray, T.K. (4)</td>
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<td>Mancusi (11)</td>
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<td>Dorling, P.R. and LePage, R.N. (17)</td>
<td>15.0-18.0</td>
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<tr>
<td>Nambu, Z. and Terayama, H. (10) using the method of Ray (4)</td>
<td>16.9</td>
</tr>
<tr>
<td>Scarpulla (12)</td>
<td>21.0</td>
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<td>Nambu, Z. and Terayama, H. (10) using the method of Lesko et al (20)</td>
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<td>Berman, H.M. (39)</td>
<td>31.6**</td>
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<tr>
<td>Takeuchi, M. and Terayama, H. (19)</td>
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All mediums for homogenization were hypotonic

**medium used for homogenization was isotonic
Electrophoretic scan obtained by electrophoresis (2.5% polyacrylamide, 0.5% agarose gels) for 1.25 hours of 7 ug plasma membrane RNA. Assay conditions are described in the text.
Electrophoretic scans obtained by electrophoresis (2.5% polyacrylamide, 0.5% agarose gels) for 1.25 hours of TOP: 7 ug plasma membrane RNA and 7 ug E. coli ribosomal RNA and BOTTOM: 7 ug E. coli ribosomal RNA. Assay conditions are described in the text.
Electrophoretic scans obtained by electrophoresis (2.5% polyacrylamide, 0.5% agarose gels) for 1.25 hours of TOP: 7.25 ug plasma membrane RNA and 7.25 ug rat liver ribosomal RNA and BOTTOM: 7.25 ug rat liver ribosomal RNA. Assay conditions are described in the text.
the actual size of the major species of plasma membrane RNA, plasma membrane RNA was again subjected to electrophoresis with rat liver ribosomal RNA as an internal marker (Figure 3). These results demonstrate that the major species of RNA associated with the plasma membrane is 28S RNA. From the electrophoretic scans, it can be stated that plasma membrane RNA consists of a major 28S RNA species, several minor species ranging from 15-22S RNA and another minor 4S RNA species.

Base Analysis of RNA

A typical elution pattern for the separation of 2',3'-ribonucleotides on a Dowex 50-H⁺ column is shown in Figure 4. Base compositions for E. coli B transfer RNA, rat liver ribosomal RNA and plasma membrane RNA are given in Tables II-A and II-B. The column was standardized according to the procedure of Katz and Comb (31), using E. coli B transfer RNA as the standard. As can be seen from Table II-B, the base compositions of both plasma membrane RNA and rat liver ribosomal RNA are similar.

Effect of RNAse and NaCl on Intact Plasma Membranes

Intact plasma membranes were incubated in the presence of 0.066 ug pancreatic RNAse. In preliminary experiments, 0.033 ug pancreatic RNAse was capable of digesting 220 ug of RNA, under the same conditions used (data not shown). It is, therefore, feasible that the total RNA in the plasma membrane (which is calculated to be approximately 220 ug) can be digested with
FIGURE 4
ELUTION PATTERN FOR THE SEPARATION OF 2',3'-RIBONUCLEOTIDES ON A DOWEX 50-H⁺ COLUMN

Column was washed with 3 N HCl, water until pH 4 and equilibrated with 15 ml 0.05 N HCl. Hydrolysed RNA was placed on the column and separated. Assay conditions are described in the text.
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<th>TYPE OF RNA</th>
<th>% UMP</th>
<th>% GMP</th>
<th>% AMP</th>
<th>% CMP</th>
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<td>E. coli B</td>
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<td></td>
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<tr>
<td>transfer RNA</td>
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<td>33.5</td>
<td>17.7</td>
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<td></td>
<td>21.3</td>
<td>34.3</td>
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<td>Rat liver</td>
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**Not used to determine average

Assay conditions are described in the text.
TABLE II-B
RNA BASE COMPOSITION AVERAGES

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<tr>
<th>TYPE OF RNA</th>
<th>% UMP</th>
<th>% GMP</th>
<th>% AMP</th>
<th>% CMP</th>
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<td>E. coli B transfer RNA (reported by Katz and Comb (31))</td>
<td>20.0</td>
<td>33.7</td>
<td>18.2</td>
<td>28.1</td>
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<tr>
<td>E. coli B transfer RNA</td>
<td>21.7</td>
<td>33.3</td>
<td>18.4</td>
<td>26.6</td>
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<tr>
<td>(± 0.19) (± 0.66) (± 0.42) (± 0.59)</td>
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<td></td>
</tr>
<tr>
<td>Rat liver ribosomal RNA (reported by Shapot et al (14))</td>
<td>20.6</td>
<td>31.7</td>
<td>20.0</td>
<td>27.7</td>
</tr>
<tr>
<td>Rat liver ribosomal RNA</td>
<td>26.6</td>
<td>34.1</td>
<td>17.1</td>
<td>23.0</td>
</tr>
<tr>
<td>(± 0.81) (± 0.62) (± 0.30) (± 0.07)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma membrane RNA</td>
<td>23.0</td>
<td>32.5</td>
<td>19.9</td>
<td>23.6</td>
</tr>
<tr>
<td>(± 1.40) (± 0.64) (± 0.93) (± 1.02)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given ± standard error
Assay conditions are described in the text.
the addition of 0.066 ug pancreatic RNAse. The effect of RNAse on intact plasma membranes is shown in Table III. These results clearly illustrate that RNAse removes 23.2% of the RNA from the membrane, while removing only 10.4% of the membrane proteins.

Intact plasma membranes were next incubated in the presence of varying concentrations of NaCl, and the results are summarized in Table IV and Figure 5. There is virtually no loss of RNA with the removal of 17.5-22.3% of membrane proteins (the plasma membranes were in a final concentration of 0.075 M and 0.15 M NaCl, respectively). However, when the plasma membranes are placed in a final concentration of 0.30 M NaCl, there is a loss of 63.2% of the RNA, while removing 25.7% of the membrane proteins.

There was a loss of plasma membrane material after each assay. It is unknown where or how the membrane material loss occurred.
### TABLE III

**EFFECT OF RNASE ON INTACT PLASMA MEMBRANES**

<table>
<thead>
<tr>
<th></th>
<th>ug RNA</th>
<th>% protein loss</th>
<th>% membrane recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ RNase</td>
<td>9.26</td>
<td>10.4</td>
<td>61.5</td>
</tr>
<tr>
<td>Control</td>
<td>12.05</td>
<td>14.8</td>
<td>49.0</td>
</tr>
</tbody>
</table>

Results obtained from a typical assay

Intact plasma membranes were incubated for one hour at room temperature in the presence of 0.073 ug/ml pancreatic RNAse. Assay conditions are described in the text.
TABLE IV
EFFECT OF NaCl ON INTACT PLASMA MEMBRANES

<table>
<thead>
<tr>
<th>Concentration NaCl (M)</th>
<th>ug RNA</th>
<th>% protein loss</th>
<th>% membrane recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.075</td>
<td>11.05</td>
<td>17.5</td>
<td>63.7</td>
</tr>
<tr>
<td>0.15**</td>
<td>12.53</td>
<td>22.3</td>
<td>54.9</td>
</tr>
<tr>
<td>0.30</td>
<td>5.14</td>
<td>25.7</td>
<td>54.6</td>
</tr>
<tr>
<td>Control**</td>
<td>13.60</td>
<td>13.5</td>
<td>57.7</td>
</tr>
</tbody>
</table>

Results obtained from a typical assay

**average of two preparations

Intact plasma membranes were incubated for one hour at room temperature in the presence of varying final concentrations of NaCl. Assay conditions are described in the text.
Intact plasma membranes were incubated for one hour at room temperature in the presence of varying concentrations of NaCl. Assay conditions are described in the text.
DISCUSSION

RNA has been shown to be associated with the plasma membrane of rat liver cells. Purity of the plasma membrane is an important factor. The membrane preparation that was used in these experiments has been previously demonstrated to be at least 95% pure by enzymatic assays and electron microscopy (12).

In recent studies, transfer RNA (4S) was found to be associated with the plasma membrane. The molecular weight of other species of RNA associated with the plasma membrane was unknown. Electrophoresis of RNA on 2.5% polyacrylamide gels (strengthened with 0.5% agarose) can easily separate RNA species, based on two factors: molecular weight and charge.

Plasma membrane RNA with E. coli ribosomal RNA (23S, 16S and 5S) or rat liver ribosomal RNA (28S, 18S and 5S) as internal markers were electrophoresed. It was demonstrated that 7 ug of RNA can easily be distinguished on the gels. It was shown that plasma membrane RNA consists of a major 28S RNA species, several minor 15-22S RNA species and a minor 4S RNA species. These results confirm the finding of transfer RNA associated with the plasma membrane (11,12).

The results obtained from the RNA base analysis indicates that plasma membrane RNA is similar to ribosomal RNA, in terms of base composition. Plasma membrane RNA is also similar to rat liver transfer RNA, in terms of base composition (base composition of rat liver transfer RNA: U:22.2% (includes 4.0% U), G: 29.2%, A:19.7%, C:28.8%)(40). These results to not imply
that plasma membrane RNA is an artifact from the cytosol, but rather is not unique to the plasma membrane.

Problems were encountered during the separation of the ribonucleotides on the Dowex column. Phenol (which was used for RNA extraction) was retained by the column, and removed by reprecipitation of the RNA in 95% ethanol.

Purity of the plasma membrane is of the utmost importance. Previous studies have shown that this plasma membrane preparation is at least 95% pure. The results of the RNA base analysis demonstrate no difference between plasma membrane RNA, ribosomal RNA and transfer RNA. Previous studies (11) have shown that lysyl- and arginyl- transfer RNA are chromatographically similar in the cytoplasm and the plasma membrane. These results give little, if any, support that RNA is an integral constituent of the plasma membrane. The major concern in this investigation was contamination of the plasma membrane with ribosomes (ribosomal RNA). The results obtained from electrophoresis of plasma membrane RNA demonstrate the presence of 28S RNA but not 18S RNA. Therefore, the plasma membrane is at least 95% pure, if not totally pure, in terms of intact ribosomes.

The physical association of the plasma membrane RNA was studied and it was demonstrated that when RNase is added to plasma membranes, there is a loss of 23.2% of the RNA, while 10.4% of the membrane proteins are released. When NaCl (final concentration of 0.15 M) is added to plasma membranes, there is virtually no loss of RNA, while 17.5-22.3% of the membrane
proteins are released. These results may indicate that some of the plasma membrane RNA is protruding from the plasma membrane but is somehow attached to the plasma membrane, as it is not removed by 0.15 M NaCl. It is unknown whether the RNA protrudes from the cytoplasmic side or exterior side of the plasma membrane. When plasma membranes are incubated in 0.30 M NaCl (final concentration), there is a loss of 63.2% of the RNA, while only 25.7% of the membrane proteins are released. Since the amount of protein released with the addition of 0.30 M NaCl is comparable to the amount of protein released with the addition of 0.15M NaCl, it must be concluded that under high ionic strengths of NaCl, RNA is released from the plasma membrane. The species of RNA released is unknown.

A perplexing question remains: How is this RNA associated with the plasma membrane? It has been shown that high concentrations of salt removes ribosomes from the rough endoplasmic reticulum (RER)(32). It has also been demonstrated that the large 60S subunit of the ribosome binds to the "receptor site" on the RER (33,34). If these findings are taken into consideration, the results obtained in this experiment would suggest that 28S RNA is being removed by the addition of 0.30 M NaCl to plasma membranes. This accounts for only 63.2% of the RNA in the plasma membrane, the location of the other 36.8% of the RNA is speculation. It may be in the form of a liporibonucleoprotein complex as described by Shapot and Davidova (5-7).

As mentioned previously the plasma membrane is at least 95% pure in terms of intact ribosomes. However, it is unknown
if the plasma membrane is 95% pure in terms of the 60S ribosomal subunit. During the plasma membrane preparation the ribosomes may have disassociated into its subunits and the 60S subunit may have attached (or remained attached) to the plasma membrane. This would account for the 28S RNA and not the 18S RNA as demonstrated by electrophoresis.

To date, there has been no assignment of biological function for the plasma membrane RNA. Several hypothesis have been postulated. Warren and Glick (35) have demonstrated that protein synthesis occurs on the plasma membrane. However, this observation may be due to the contamination of the plasma membrane with ribosomes. Plasma membrane RNA, specifically transfer RNA, has been implicated in the transport of amino acids across the plasma membrane (36). There is evidence that the RNA may be an integral part of the plasma membrane, in the form of a liporibonucleoprotein complex (7). Plasma membrane RNA may act as recognition sites for cell to cell interactions by complimentary reactions between RNAs of cell surfaces (37). Recently, Rothman and Lenard (38) have suggested that ribosomes may bind to the plasma membrane.

Rothman and Lenard's hypothesis was indirectly tested by incubating the plasma membranes with NaCl. From what has been mentioned previously, this model is feasible if two assumptions are taken: (1) the RNA released is 28S RNA and (2) the RNA is released from the cytoplasmic side of the plasma membrane. The results obtained from the RNAse incubation disagrees with the above model. This, however, does not rule out this model as
other species of RNA may be located on the periphery of the plasma membrane. It does, however, assume that the 28S RNA is not accessible to the RNAse (i.e. the RNA is surrounded by ribosomal proteins) and therefore the plasma membrane is contaminated with the 60S ribosomal subunit. The RNAse data may also indicate, if the plasma membranes are assumed to be 95% pure in terms of the 60S ribosomal subunit, that the RNA (assumed to be 28S) is an integral part of the plasma membrane and is released under high ionic strengths of NaCl.

To elucidate some questions about the function(s) and physical association of the RNA with the plasma membrane, more experiments are needed. Such experiments as the actual determination of what species of RNA is released from the plasma membrane by treatment with NaCl is crucial a determination of the actual species of RNA(s) and its physical association with the plasma membrane. The injection of radioactive materials (uridine and/or amino acids) into the rat and isolating the plasma membrane may determine the purity of the plasma membrane in terms of ribosomal and ribosomal subunit contamination. This may be accomplished by the amount of radioactivity associated with the plasma membrane (ribosomal contamination). Extraction of ribosomes (if attached) from the plasma membrane fraction and ultimately the separation of the ribosomal subunits on sucrose gradients may lead to the amount of ribosomal subunit contamination. Incubation of the ribosomal subunits and the incubation of ribosomal RNA (28S and 18S) with the plasma membrane may lead to a better understanding of ribosomal
attachment to the plasma membrane, if it does indeed bind. It may also be helpful if the cytoplasmic side of the plasma membrane can somehow be distinguished from the exterior side, to determine the RNAs actual physical association with the plasma membrane.

The results obtained from these experiments show basically three types of RNA associated with the plasma membrane; (1) a major 28S species, (2) several minor 15-22S species and (3) a minor 4S species. Possible reasons for each of these species associated with the plasma membrane are given in Table V.

The actual function(s) and physical association of the RNA associated with the plasma membrane is still speculation. Although these experiments alleviated the problem slightly, more work is needed to resolve these problems associated with plasma membrane RNA.
TABLE V
POSSIBLE REASONS FOR PLASMA MEMBRANE RNA SPECIES

28S species (major)
- may bind ribosomes (60 subunit) to the cytoplasmic side of the plasma membrane
- may act as a receptor site for various molecules or cells, if located on the exterior side of the plasma membrane
- may act as an integral structural component of the plasma membrane
- may be an artifact due to the binding of the 60S ribosomal subunit

15-22S species (minor)
- may act as an integral structural component of the plasma membrane (different molecular weights due to what phospholipid the RNA is bound to)
- may act as a receptor site for various molecules or cells
- may be a degradation product of 28S RNA

4S species (minor)
- may act as a receptor site for various molecules or cells
- may transport amino acids across the plasma membrane
- may act as an integral structural component of the plasma membrane
- may be present if ribosome attaches and protein synthesis occurs
- may be an artifact due to isolation procedures
BIBLIOGRAPHY