Nuclear Ribonucleoprotein Complexes Containing U1 and U2 RNA†

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ABSTRACT: Nuclear ribonucleoprotein (RNP) complexes that contain the U1 and U2 RNA of chromatin of Novikoff hepatoma cells were extracted with 0.01 *M* Tris-HCl (pH 8.0) after the nuclei were initially washed with 0.075 *M* NaCl and 0.025 *M* EDTA (pH 8.0). These RNP complexes were purified by chromatography on Sepharose 6B columns and centrifugation on sucrose density gradients. The identity of the U1 and U2 RNA in these particles was established by their electrophoretic mobility in polyacrylamide gels and their T_1 RNase fingerprints which were identical with those of authentic U1 and U2 RNA (R. Reddy et al. (1974), *J. Biol. Chem. 249,* 6486–6494; H. Shibata et al. (1974), *Mol. Cell. Biochem. 4,* 3–19). The nuclear ribonucleoproteins had a buoyant density of 1.47 g/ml in CsCl gradients. Two-dimensional polyacrylamide gel electrophoresis of their proteins showed these RNP complexes contain 10 polypeptide spots, of which two are phosphorylated in vivo.

The low molecular weight nuclear RNA (LMWN)[1] species of Novikoff hepatoma ascites tumor cells include six groups of RNAs, the 4S, 4.5S, 5S, U1, U2, and U3 RNA (Ro-Choi and Busch, 1974a). The function of most of these LMWN RNAs is not defined but 4S RNA is mainly tRNA, 5S RNA is incorporated into ribosomal precursor particles, U3 is nucleolus specific, and U1, U2, and 4.5S RNA are located in chromatin (Ro-Choi and Busch, 1974b). The U1 and U2 RNAs which have chain lengths of 171 and 196, respectively, have unique nucleotide sequences (Reddy et al., 1974; Shibata et al., 1974, 1975) including the common feature of a highly methylated 5′ terminus which contains the unusual nucleoside $m_3^{2,2,7}G^1$ in pyrophosphate linkage to the 5′ portion of the molecule (Ro-Choi et al., 1975). These RNA species of chromatin have been found in mammals, amphibia, and birds (Knight and Darnell, 1967; Dingman and Peacock, 1968; Weinberg and Penman, 1968; Rein and Penman, 1969).

In preliminary studies on sedimentation of nuclear RNP particles, it was observed that fractions containing U1 and U2 RNA sedimented more rapidly than free U1 and U2 RNA suggesting the possibility that these RNA species exist in RNP complexes. These results support the earlier suggestion that some LMW RNA exist in protein complexes (Rein, 1971; Enger et al., 1974). These present studies show that small nuclear RNP complexes exclusively containing U1 and U2 RNA were readily purified on Sepharose 6B columns and sucrose density gradients.

Materials and Methods

Animals. The Novikoff hepatoma ascites cells were maintained in male Holtzman rats for 6 days, at which time the rats were sacrificed and the abdominal fluid was drained into containers kept on ice.

Isotopes. RNA was labeled in vivo for 25 hr with [^{32}P]orthophosphate by injecting 40–60 mCi intraperitoneally in two doses with an interval of 8 hr. For in vitro labeling, about 20 g of cells was incubated with carrier-free isotope (500 mCi) according to procedures described by Mauritzen et al. (1971).

Particle Preparation. Novikoff hepatoma ascites tumor cells were harvested and washed twice with 0.05 *M* Tris-HCl buffer (pH 7.6) containing 0.025 *M* KCl and 5 m*M* $MgCl_2$ (TKM) to remove the red blood cells. The nuclei were prepared by a procedure employing Nonidet P-40 which markedly decreased cytoplasmic contamination.[2] The washed cells were suspended in 10 volumes of 0.01 *M* Tris-HCl, 0.01 *M* NaCl, 1.5 m*M* Mg^{2+}, and 0.5% Nonidet P-40 at pH 7.6, homogenized with a pestle rpm of 600 and centrifuged at 3000*g* for 15 min. The pellet was resuspended in the TKM buffer and the procedure was repeated. The nuclei prepared by this procedure are relatively free of cytoplasmic tags; they were extracted twice with 0.075 *M* NaCl and 0.025 *M* EDTA at pH 8, then three times with 10 volumes of 0.01 *M* Tris-HCl buffer at pH 8.0 containing 1 m*M* phenylmethanesulfonyl fluoride ($PhCH_2SO_2F$), and finally twice with 10 volumes of STM 8 buffer (0.01 *M* Tris-HCl (pH 8.0) containing 1 m*M* Mg^{2+} and 0.14 *M* NaCl). The Tris wash was concentrated on an Amicon PM 30 ultrafilter and passed through a 3 × 75 cm column of Sepharose 6B equilibrated with STM 8 buffer containing 1 m*M* $PhCH_2SO_2F$. The flow through fractions were pooled, concentrated on an Amicon apparatus, and centrifuged for 16 hr at 24,000 rpm on 5–30% linear sucrose density gradients in STM 8 buffer. The gradients were analyzed and fractions were collected with the aid of an ISCO Model D fractionator.

The RNP in the gradient was precipitated with 2.5 vol-

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[1] Abbreviations used are: $m_3^{2,2,7}G$, N^2,N^2-dimethyl-7-methylguanosine; LMWN, low molecular weight nuclear RNA; TKM buffer, 0.05 *M* Tris-HCl, 0.025 *M* KCl, and 5 m*M* $MgCl_2$; $PhCH_2SO_2F$, phenylmethanesulfonyl fluoride; STM 8 buffer, 0.01 *M* Tris-HCl (pH 8.0), 1 m*M* Mg^{2+}, 0.14 *M* NaCl; RNP, ribonucleoprotein.

[2] To ascertain whether nuclei prepared by two different procedures contain these particles, sucrose nuclei prepared by methods previously described (Busch and Smetana, 1970) were also extracted in the same way. The particles obtained by this procedure were essentially the same as those obtained with the Nonidet P-40 method employed for most of these studies.

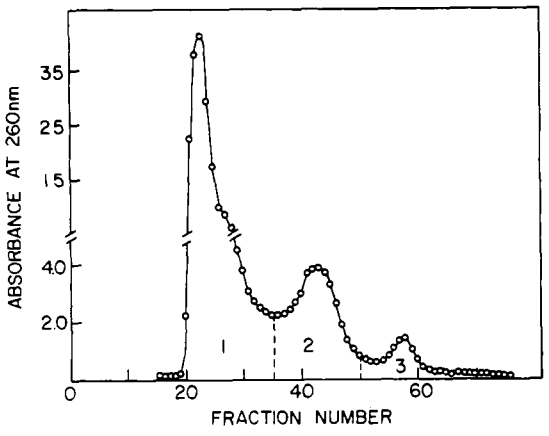

FIGURE 1: Elution pattern of 0.01 *M* Tris-HCl (pH 8.0) extract of nuclei on Sepharose 6B (3 × 75 cm). The sample was eluted with 0.01 *M* Tris-HCl (pH 8.0), 0.14 *M* NaCl, 1 m*M* $MgCl_2$, and 1 m*M* $PhCH_2SO_2F$; 5-ml fractions were collected.

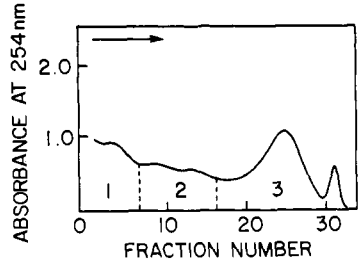

FIGURE 2: Sucrose density gradient profile of the Sepharose 6B flow-through fraction of 0.01 *M* Tris-HCl (pH 8.0) extract of Nonidet P-40 nuclei. The centrifugation was done in 5-30% sucrose gradients containing 0.01 *M* Tris-HCl (pH 8.0), 0.14 *M* NaCl, and 1 m*M* $MgCl_2$, at 24,000 rpm for 16 hr in a SW-27 rotor. The arrow shows the direction of sedimentation. The ribonucleoproteins in fractions 1, 2, and 3 were precipitated with 2.5 volumes of ethanol and RNA was extracted with phenol.

umes of ethanol containing 2% potassium acetate and the RNA was extracted by the sodium dodecyl sulfate–phenol procedure (Higashi et al., 1966; Hodnett and Busch, 1968; Ro-Choi et al., 1970). The RNA was reprecipitated with ethanol after dissolving in 1 m*M* EDTA.

Polyacrylamide Gel Electrophoresis of RNA. Electrophoresis was performed either in cylindrical analytical gels (9.5 cm long) with an inner diameter of 5 mm or on slab gels (15 cm × 30 cm × 3 mm). The gel solutions contained 8 or 10% acrylamide, 0.25% bisacrylamide, 0.05% Temed, and 0.1% ammonium persulfate. The slab gels contained a 5-cm stopper gel, a 25-cm running gel, and a 1–2-cm 5% polyacrylamide stacking gel (Ro-Choi et al., 1973). With slab gels, electrophoresis was carried out for 40 hr with a current of 40 mA at a voltage of 300 V in a buffer containing 0.02 *M* Tris-acetate (pH 7.2), 0.02 *M* NaCl, and 0.04 *M* EDTA. For analytical gels, electrophoresis was carried out at a constant current of 4 mA/tube. The gels were stained with Methylene Blue for 1 hr and the excess dye was destained by leaving them in running water. The gels were scanned at 590 nm in a Gilford gel scanner.

Purification of U1 and U2 RNAs. The 4–8S RNA was prepared as described previously by centrifugation on 5–40% sucrose density gradient and precipitated twice with 2 volumes of ethanol (Ro-Choi et al., 1973). The U1 and U2 RNAs were separated on 10% polyacrylamide slab gels. The bands were excised and the RNA was extracted from the gels with 0.2% NaCl and 6 *M* urea at neutral pH. The RNA was concentrated by adsorbing it on DEAE-Sephadex A-25 columns and eluting it with 30% triethylammonium bicarbonate. After neutralizing with acetic acid, the RNA was precipitated with 2.5 volumes of ethanol.

Digestion of RNA by T_1 RNase. The RNA was air dried and enzyme was added directly in a volume of 5–10 μl (enzyme/substrate ratio, 1:20). Digestion was carried out at 37°C for 30 min.

Fractionation of Oligonucleotides Produced by T_1 RNase Digestion. The two-dimensional method of Sanger and Brownlee (1967) was employed. Cellulose acetate strips (3 × 90 cm) were wet with 5% acetic acid, 0.1% pyridine (pH 3.5), and 7 *M* urea. The digest was applied directly to the strips. Electrophoresis was carried out at a constant voltage of 2.5 kV. When the blue dye (Xylene Cyanol F.F.) migrated 12–15 cm, the sample was transferred to DEAE-cellulose paper (42 × 80 cm). The second electrophoresis was carried out on DEAE-cellulose paper in the 7% formic acid with a constant current of 100 mA/sheet. Electrophoresis was carried out until the blue dye marker migrated 15–20 cm.

Isopycnic Banding of Fixed RNP Particles Containing U1 and U2 RNA in CsCl Density Gradients. The particulate fraction from sucrose gradients containing U1 and U2 RNA was dialyzed overnight against 0.01 *M* triethanolamine-HCl (TEA) buffer (pH 7.8) containing 0.14 *M* NaCl and was concentrated in an Amicon apparatus. The sample was then fixed by the addition of sodium bicarbonate neutralized formaldehyde to a final concentration of 6% (4°C). After overnight fixation, it was dialyzed against the TEA buffer for 8 hr; 2.5 ml of sample containing 1 g of CsCl was then layered over 2.5 ml of buffer containing 2 g of CsCl (Brunk and Leick, 1969). Centrifugation was for 48 hr at 40,000 rpm in an SW 65 rotor (5°C). The gradients were fractionated using an ISCO fractionator.

Extraction of Proteins from Particles. The particle fractions from the sucrose density gradient centrifugation were centrifuged at 200,000*g* for 15 hr and the pellets were suspended by mild homogenization in minimal volumes of 66% acetic acid containing 0.1 *M* $MgCl_2$. The suspension was stirred overnight and centrifuged at 10,000*g* for 30 min. The supernatant was dialyzed against 0.9 *N* acetic acid and concentrated by dialysis against solid sucrose. With this method, 33% of total protein in the RNP fraction was solubilized.

Two-Dimensional Gel Electrophoresis of Proteins. The proteins from RNP fraction were separated by two-dimensional gel electrophoresis (Busch et al., 1974; Orrick et al., 1973).

Results

Isolation of U1, U2 RNA–Protein Complexes from Isolated Nuclei. The 0.01 *M* Tris-HCl extract (pH 8) obtained from Novikoff hepatoma cell nuclei was concentrated by filtration through an Amicon membrane and passed through a Sepharose 6B column (3 × 75 cm) to remove low molecular weight components (Figure 1). The flow-through fraction (fraction 1) which contained all the U1 and U2 RNA was concentrated and separated on a 5–30% sucrose density gradient (Figure 2). The RNP complexes in the fractions were precipitated with ethanol and the phenol extracted RNA was analyzed on 8% polyacrylamide gels (Figure 3). Fraction 1 contained 4S, 5S, U1, and U2 RNA in approximately equal amounts. The U1 and U2 RNA were mainly in fraction 2. Fraction 3 contained mainly 5S

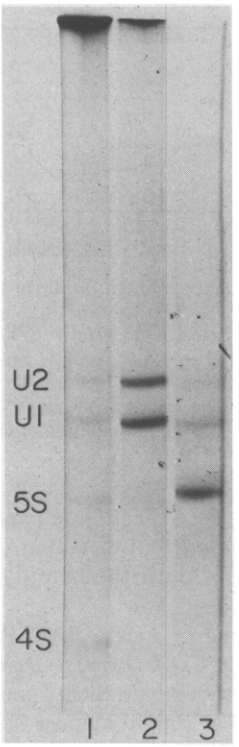

FIGURE 3: Polyacrylamide gel electrophoretic pattern of RNA extracted from fractions 1, 2, and 3 of Figure 2. The gels were stained with 0.2% Methylene Blue.

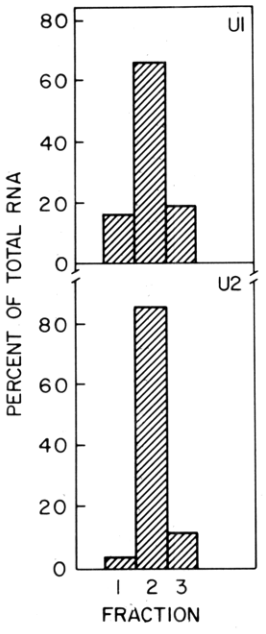

FIGURE 4: Distribution of U1 and U2 RNA in fractions 1, 2, and 3 from Figure 3. The stained gels were scanned at 590 nm and area under each peak was determined by weighing the paper. The total area under each peak was calculated by the equation: (area) (amount of RNA in the fraction)/amount of RNA loaded on the gel.

RNA and a small amount of U1 and U2 RNA. The distributions of U1 and U2 RNA in fractions 1, 2, and 3 are shown in Figure 4. The bulk of the U1 and U2 RNA is in fraction 2 of the gradient which contained 64% of U1 and 83% of U2 RNA in the 0.01 *M* Tris-HCl (pH 8) fraction.

Distribution of RNA in Various Nuclear Extracts. RNA was prepared from the cytoplasmic fraction and nuclear extracts were obtained with 0.075 *M* NaCl and 0.025 *M*

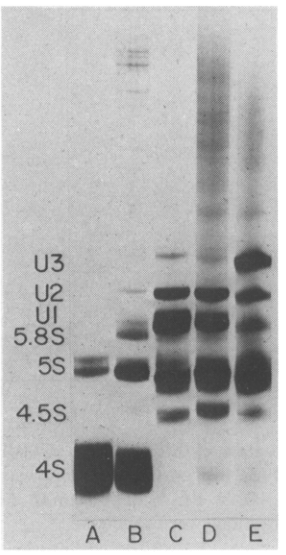

FIGURE 5: Polyacrylamide gel electrophoretic pattern of LMW RNA from fractions: (A) cytoplasm; (B) saline–EDTA, pH 8 extract; (C) 0.01 *M* Tris-HCl, pH 8.0 extract; (D) 0.01 *M* Tris-HCl, pH 8.0, 0.14 *M* NaCl, and 1 m*M* $MgCl_2$ extract; (E) residue. The gels were stained with 0.2% Methylene Blue. As shown in Figure 6, most of the U1 and U2 RNA were extracted in (C).

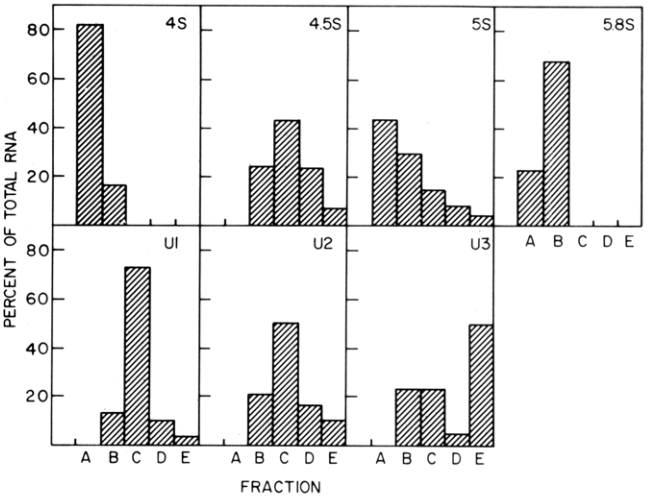

FIGURE 6: Percent distribution of the LMW RNA in the nuclear extracts. The stained gels from Figure 5 were scanned at 590 nm and the area under each peak was determined by weighing the paper. A standard analysis was performed to establish linearity of staining and quantity of RNA in the range of the bands from the stained gels. The total area under each peak was calculated by an equation: (area) (amount of RNA in the extract)/amount of RNA loaded on the gel. (A) Cytoplasm; (B) saline–EDTA, pH 8 extract; (C) 0.01 *M* Tris-HCl, pH 8.0 extract; (D) 0.01 *M* Tris-HCl (pH 8.0), 0.14 *M* NaCl, and 1 m*M* $MgCl_2$ extract; (E) residue.

EDTA, 0.01 *M* Tris-HCl (pH 8), STM 8 buffer (see Materials and Methods), and the nuclear residue after these extractions. After sucrose density gradient centrifugation to obtain the low molecular weight RNA, these fractions were analyzed on 8% acrylamide gels (Figure 5). The distribution of each of the LMW RNAs in these fractions is shown in Figure 6. The bulk of the 4S RNA was found in the cytoplasmic fraction (Figure 6A) and the remainder was extracted by the saline–EDTA solution (Figure 6B). By contrast, the U1 and U2 RNA were preferentially extracted with 0.01 *M* Tris-HCl (pH 8) (Figure 6C and D) which contained 73% of the U1 RNA and 50% of the U2 RNA.

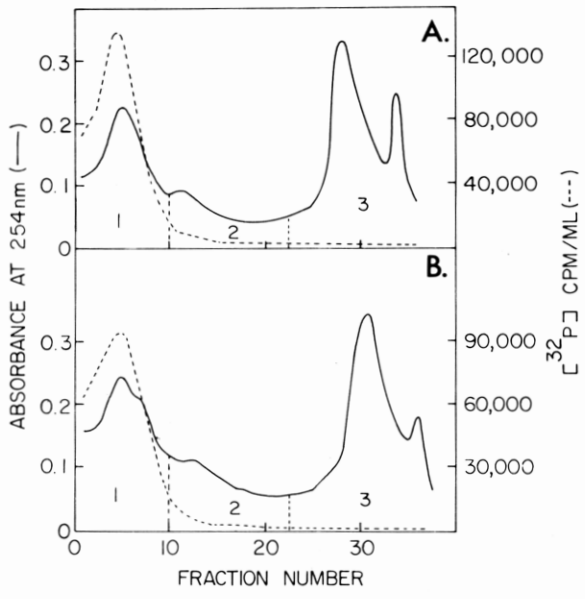

FIGURE 7: Sucrose density gradient profile of a nucleoplasmic extract. ^{32}P-labeled U1 or U2 RNA was added to the sucrose-Ca^{2+} nuclei (Busch, 1967) which were then sonicated in 0.25 *M* sucrose containing 1 m*M* Mg^{2+}. The post-nucleolar supernatant was centrifuged at 250,000*g* for 16 hr and the pellet was extracted with 0.01 *M* Tris-HCl buffer containing 1 m*M* Mg^{2+} and 0.14 *M* NaCl at pH 8.0 (STM 8). The extract was centrifuged at 10,000*g* for 30 min and the supernatant was layered on 5–30% sucrose gradients made up in STM 8 buffer. The centrifugation was for 17 hr in a SW-27 rotor at 24,000 rpm. (A) ^{32}P-labeled U1 RNA was mixed with unlabeled nuclei. (—) Optical density, (- - -) radioactivity.

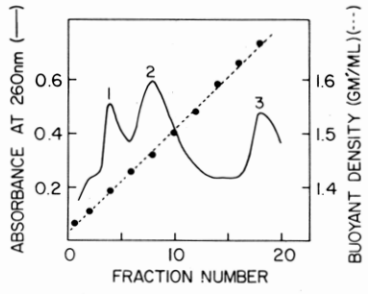

FIGURE 8: Isopycnic banding of formaldehyde-fixed RNP fraction containing U1 and U2 RNA in a CsCl gradient. Centrifugation was for 48 hr at 40,000 rpm in the SW-65 rotor.

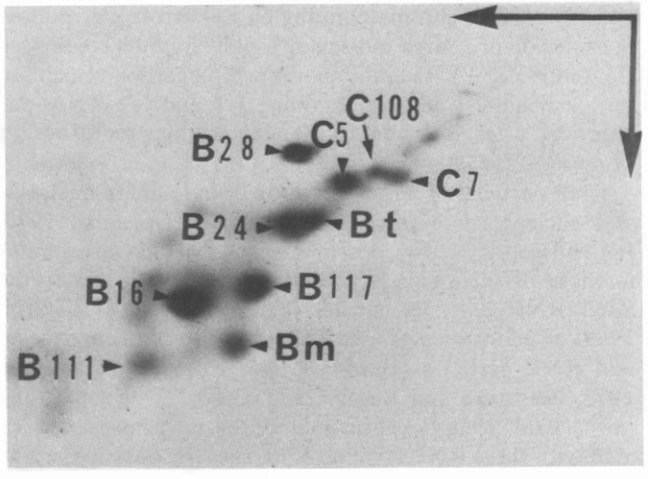

FIGURE 9: Two-dimensional polyacrylamide gel electrophoresis of protein from fraction 2 of Figure 2. First dimension was on disc gels of 10% acrylamide, 4.5 *M* urea, and 0.9 *N* acetic acid at 120 V for 5.5 hr. For the second dimension, a 12% acrylamide–0.1% dodecyl sulfate slab gel was run for 15 hr at 50 mA/slab. Gels were stained with Coomassie Brilliant Blue R.

Most of the U3 RNA remained in the residue fraction (Figure 6E), in agreement with previous findings that U3 RNA is localized to the nucleoli (Ro-Choi and Busch, 1974b).

To ascertain whether the RNA extracted from the RNP fractions was authentic U1 and U2 RNA, the T_1 RNase digestion products were compared with known samples of U1 and U2 RNA. The results indicated that the U1 and U2 RNA present in these RNP particles were identical with those sequenced earlier (Reddy et al., 1974; Shibata et al., 1974).

Characterization of Ribonucleoproteins Containing U1 and U2 RNA. A number of investigators have noted that RNA sediments rapidly when added to cell homogenates because of nonspecific association with the positively charged proteins (Girard and Baltimore, 1966; Ovchinnikov et al., 1968; Spirin, 1969). To determine if such association resulted in the rapid sedimentation of the U1 and U2 RNA, ^{32}P-labeled U1 or U2 RNA was added to unlabeled nuclei prior to extraction; the nuclei were then sonicated. Since the labeled exogenous U1 or U2 RNA did not cosediment with the particle fraction containing U1 and U2 RNA (Figure 7),[3] the binding is not likely to be artifactual.

Cesium Chloride Isopycnic Analysis. The nature of the particles containing U1 and U2 RNA was further investigated by isopycnic analysis following formaldehyde fixation (Spirin, 1972). The ribonucleoproteins of fraction 2 of the sucrose density gradient banded with a buoyant density of 1.47 g/ml (Figure 8, peak 2) which indicates that the particle contains 64% protein and 36% RNA (Spirin, 1972).[4] The material in peaks 1 and 3 banded with buoyant densities of 1.38 and 1.67 suggesting that they contain protein and RNA, respectively, presumably from dissociated RNP particles.

Resolution of the Protein Components. Proteins were extracted from the fraction 2 of the RNP sucrose density gradient (Figure 2) as described in Materials and Methods. The pattern of two-dimensional gel electrophoresis of protein is shown in Figure 9. Ten polypeptide spots in the B and C region of the gel were consistently detected; of these, six were major and four were minor spots. In studies with ^{32}P, spots B16 and C5 were found to be consistently phosphorylated. Comparisons of these patterns with corresponding patterns for proteins of ribosomal subunits (Prestayko et al., 1974) indicate that none of these proteins are ribosomal proteins.

Discussion

Small RNP particles containing low molecular weight RNA have been found by Rein (1971) in mitotic cells and by Enger et al. (1974) in Chinese hamster ovary cells. In the present studies, small nuclear ribonucleoprotein com-

[3] Since some of the U1 or U2 RNA was degraded during the procedure and some remained in the supernatant fraction after centrifugation at 250,000*g* for 16 hr, it would appear that the protein portion of the particle protects these RNA species from the exogenous RNases.

[4] The protein and RNA content were calculated from the buoyant density using an equation: percent protein = $(1.85 - \rho)/0.006$, where ρ = buoyant density and percent RNA = 100 − percent protein.

plexes containing specific U1 and U2 RNA were extracted from nuclei with 0.01 *M* Tris-HCl (pH 8.0) and purified on Sepharose 6B and sucrose density gradients. The identity of the U1 and U2 RNA in the particles was established by their characteristic mobility on the gels and by T_1 RNase fingerprints (Reddy et al., 1974; Shibata et al., 1974). The existence of these RNAs in RNP complexes was demonstrated by their buoyant density of 1.47 g/ml which indicates that they contain 66% protein and 34% RNA (Spirin, 1972).

That the RNA is bound to the protein was shown by the finding that (1) when phenol extracted U1 or U2 RNA was added to the nuclei prior to disruption, the added RNA did not cosediment with the U1 and U2 RNA in the particles and (2) column chromatography on Sepharose 6B resulted in exclusion of a large molecular weight fraction containing U1 and U2 RNA. In addition, the sedimentation velocity of the particulate fraction containing U1 and U2 RNA was identical after isolation in isotonic or hypotonic buffers (Figures 2 and 7).

RNP particles of larger size have been found in nucleolar and nucleoplasmic fractions (Busch and Smetana, 1970; Bouteille et al., 1974). Their buoyant densities are similar to those of the small RNP particles found in this study. Such RNP particles include (1) informosomes (Spirin, 1969) or informofers (Samarina et al., 1968); (2) preribosomal RNP particles (Burdon, 1971; Kumar and Warner, 1972; Prestayko and Busch, 1973); and (3) "gene regulators" (Paul, 1970). Approximately ten proteins are associated with these RNP complexes; of these, six are major and four are minor spots; two of these, C-5 and B-16, were consistently found to be phosphorylated. The role of phosphorylation in gene control mechanisms and in maturation of nuclear particles has been extensively reviewed (Teng et al., 1971; Stein and Borun, 1972; Borun and Stein, 1972; Stein and Baserga, 1972; Olson et al., 1974; Prestayko et al., 1974; Kostraba et al., 1975).

The loose attachment of U1 and U2 RNA to the chromatin along with the number of these molecules (which approximate that of the number of functional genes) and their low turnover rate suggest that they may serve as binding sites or have a regulatory role in gene function. A nuclear ribonucleoprotein particle has been reported by Liao et al. (1973) and Liang and Liao (1974) to bind to a specific steroid–receptor complex. Several models for gene regulation in higher organisms include RNA (Britten and Davidson, 1969; Georgiev, 1972) and Kanehisa et al (1972) have suggested that some low molecular weight chromatin RNA stimulated RNA synthesis in vitro. Recently, Sekeris and Niessing (1975) have reported that low molecular weight RNAs serve to maintain the integrity of RNP particles carrying HnRNA. Goldstein and Ko (1974) suggested that shuttling low molecular weight RNA concentrates in the nucleus to serve for signal transmissions in transcription and/or replication.

Some drugs produce metabolic variations in nuclear content and turnover of the LMWN RNAs (Weinberg and Penman, 1969; Howard and Stubblefield, 1972; Frederiksen and Hellung-Larsen, 1974). Preliminary studies indicated that cycloheximide, α-amanitin, and thioacetamide induced a shift in the proportions of low molecular weight RNAs of normal livers of rat or Novikoff hepatoma ascites cells. For detailed studies on the function of the LMWN RNA, it is important that the RNP complexes should be studied rather than the free RNA alone.

Acknowledgments

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Raman Spectra and Structure of Yeast Phenylalanine Transfer RNA in the Crystalline State and in Solution†

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ABSTRACT: Laser Raman spectra of yeast phenylalanine transfer RNA have been obtained in solution and in orthorhombic and hexagonal crystals. So far as one can tell from the spectra, which are identical in the two crystal forms, the molecular structure of the tRNA is not altered by differences in molecular packing in these two unit cells. In addition, the spectra of the two crystal forms show the same characteristic Raman frequencies and intensities as those of the tRNA in aqueous solution. Thus the structure of the tRNA molecule appears to be the same in the crystals and in aqueous solution. From the spectroscopic changes that result when Mg^{2+} ions are removed from the native tRNA, it is concluded that the removal of Mg^{2+} produces a partial disordering of the ribophosphate backbone of the molecule and a lowering of its melting temperature. The melting is shown to be a complex process in that the vibrations specific for adenine indicate a slightly lower melting temperature and those specific for guanine a slightly higher melting temperature than that of the ribophosphate backbone.

Detailed knowledge of the three-dimensional structure of macromolecules is obtained largely through X-ray diffraction studies of crystals. One of the central problems raised by studies of this type is the relationship between the three-dimensional structure revealed in the crystal study to the structure of the molecule in aqueous solution where the material is biologically active. During the past few years a great deal has been learned about the three-dimensional structure of yeast phenylalanine transfer RNA (tRNA^{Phe}) from X-ray diffraction studies. In the present report we make a comparison of the laser Raman spectra produced by this tRNA in the crystalline state and in a solution where it is biologically active. Since Raman spectra are sensitive to structure and molecular conformation, they represent a powerful method of comparing the molecule in these two states.

Yeast tRNA^{Phe} was used in X-ray diffraction studies because of the discovery 4 years ago that spermine complexes of this molecule produce crystals which yield high-resolution diffraction patterns (Kim et al., 1971). Over 2 years ago the electron density map at 4-Å resolution revealed that the molecules have L-shaped conformation with the acceptor and TψC stems of the familiar cloverleaf forming one arm of the L, while the dihydrouracil (D) stem and the an-

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