Multiple States of U3 RNA in Novikoff Hepatoma Nucleoli†

Paul Epstein, Ram Reddy, and Harris Busch*

ABSTRACT: U3 RNA, a capped small nuclear RNA found thus far only in the nucleolus, has been implicated in the processing and/or transport of preribosomal RNA [Busch, H., Reddy, R., Rothblum, L., & Choi, Y. C. (1982) *Annu. Rev. Biochem. 51*, 617–654]. Tris(hydroxymethyl)aminomethane (Tris) (10 mM, pH 7.0) extracts of Novikoff hepatoma nucleoli, which contained about 80% of total nucleolar U3 RNA, were analyzed by sucrose density gradient centrifugation. Approximately 65% of the U3 RNA was bound to greater than 60S preribosomal ribonucleoprotein (RNP) particles, and about 15% sedimented at less than 20 S. The association between the 65% of U3 RNA that was bound to the preribosomal RNP particles was stable up to 55 °C. About 10% of U3 RNA was base paired to preribosomal RNA after deproteinization at 22 °C. The base-paired fraction of U3 RNA was released from the preribosomal RNA by heating to 45 °C or treating with 4 M urea. These results show that of the total nucleolar U3 RNP, (a) about 55% is bound to preribosomal RNP particles primarily by protein interactions, (b) about 10% is base paired to preribosomal RNA, (c) approximately 15% sedimented slowly and consisted presumably of free U3 RNP particles, and (d) the remaining 20% of U3 RNP was not extractable using 10 mM Tris buffer. On the basis of the different association states of U3 RNP particles, a model is proposed for the binding and dissociation events which take place between U3 RNP and preribosomal RNP particles.

U3 RNA is the only capped, small nuclear RNA (snRNA)[1] reported to date to be localized to the nucleolus (Busch et al., 1982; Reddy & Busch, 1981). The extensive sequence homologies found between rat U3 RNA and the capped D2 snRNA of *Dictyostelium discoideum* (Wise & Weiner, 1980) and the identification of a U3 RNA species in dinoflagellate cells (Reddy et al., 1983b) indicate that there is a remarkable degree of evolutionary conservation of U3 RNA.

U3 RNA has been implicated in the processing and/or transport of preribosomal RNA since a portion U3 RNA is bound to preribosomal RNA by RNA–RNA base pairs (Prestayko et al., 1971; Zieve & Penman, 1976; Reddy et al., 1981; Calvet & Pederson, 1981). The correlation of U3 RNA synthesis to the synthesis and processing of preribosomal RNA (Frederiksen & Hellung-Larsen, 1974; Ro-Choi et al., 1976) and the nucleolar localization of U3 RNA (Busch et al., 1982) are consistent with this suggestion. Two models for the base pairing of U3 RNA near the cleavage sites in 32S preribosomal RNA have been proposed (Crouch et al., 1983; Bachellerie et al., 1983).

A recent report (Mount et al., 1983) indicates that site-specific binding of U1 RNP to hnRNA requires both RNA–RNA and protein–RNA interactions. The experiments described in this report indicate that U3 RNA binding to preribosomal RNP particles is similar since both RNA–RNA and RNA–protein interactions are involved. The principle interactions appear to be RNA–protein. Although 65% of U3 RNA was associated with preribosomal particles, only 10% of U3 RNA was base paired to preribosomal RNA.

Experimental Procedures

Nucleoli were isolated by sonication of Novikoff hepatoma ascites cells as described earlier (Matsui et al., 1977). ^{32}P-Labeled nucleoli were prepared in the same way as unlabeled nucleoli except that the cells were incubated for 16 with [^{32}P]orthophosphate (Mauritzen et al., 1970) prior to isolation of nucleoli.

Preribosomal RNP particles were obtained by two extractions of nucleoli with 30 volumes of 10 mM Tris (pH 7.0)/5 mM vanadyl ribonucleoside complex (VRC, Bethesda Research Laboratories, Bethesda, MD)/1 mM PMSF/0.1 mM leupeptin (Tris buffer) using a tight Dounce homogenizer. The combined Tris extracts were fractionated by sucrose density gradient centrifugation or by differential centrifugation. Sucrose density gradients (5–50%) contained 10 mM Tris (pH 7.0) and 2 mM VRC and were centrifuged for 16 h at 70000g_{av} in a Beckman SW 28 rotor. Since VRC absorbs strongly at wavelengths of 260 and 280 nm, absorbance profiles were obtained from parallel gradients in which no VRC was used in extractions or sucrose density gradient buffers. VRC was used in the extraction and gradient buffers since it reduced degradation of preribosomal RNA which otherwise obscured the gel patterns of the small nucleolar RNAs. Differential centrifugation of Tris extracts was done by centrifugation for 2 h in a Beckman Ti-40 rotor at 87000g_{av}. These centrifugation conditions pelleted the preribosomal RNP particles but less than 5% of free small RNAs or small RNP particles.

RNA was extracted at 22 °C by the sodium dodecyl sulfate–phenol method (Mauritzen et al., 1970) followed by ethanol precipitation and in most instances by digestion with 1 mg/mL proteinase K in 0.1% SDS/10 mM Tris (pH 7.0)/0.1 M NaCl for 6–12 h at 22 °C. Protein concentrations were measured by the Bio-Rad procedure. Deproteinized RNA was fractionated on 5–40% sucrose density gradients containing 0.1 M NaCl and 10 mM Tris (pH 7.0) in a Beckman SW 28 or 41 rotor for 16 h at 70000g_{av}.

Small RNAs were analyzed by polyacrylamide gel electrophoresis. Gels consisted of a 10 cm long, upper stacking gel of 5% acrylamide and a 30 cm long, running gel of 10% acrylamide. The gels contained 7 M urea and were run in Tris–borate (pH 8.3) buffer. It was necessary to remove high molecular weight RNA from samples prior to electrophoresis,

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[1] Abbreviations: snRNA(s), small nuclear RNA(s); hnRNA, heterogeneous nuclear RNA; RNP, ribonucleoprotein; VRC, vanadyl ribonucleoside complex; Tris, tris(hydroxymethyl)aminomethane; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate.

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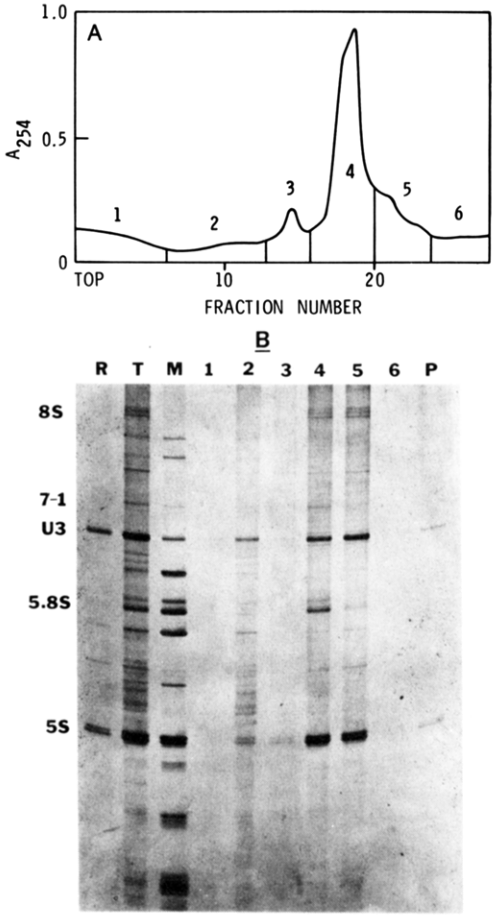

FIGURE 1: (A) Absorbance profile of a 5–50% sucrose density gradient on which the Tris extract of Novikoff hepatoma nucleoli was applied. The extraction procedure and centrifugation conditions are as described under Experimental Procedures except that no VRC was used. Gradients were collected in 1.2-mL fractions and pooled as indicated. (B) Silver-stained gel of the small nucleolar RNAs in the Tris extract (lane T) and the unextracted residue (lane R) of Novikoff hepatoma nucleoli. Lanes 1–6 and lane P show, respectively, the small nucleolar RNAs of pools 1–6 and the pellet of a sucrose gradient identical with the gradient shown in panel A except the gradient and extract contained VRC, as described under Experimental Procedures. Lane M contains 4–8S nuclear RNA. 5.8S RNA splits into two bands under these electrophoresis conditions; the lower, major band is marked.

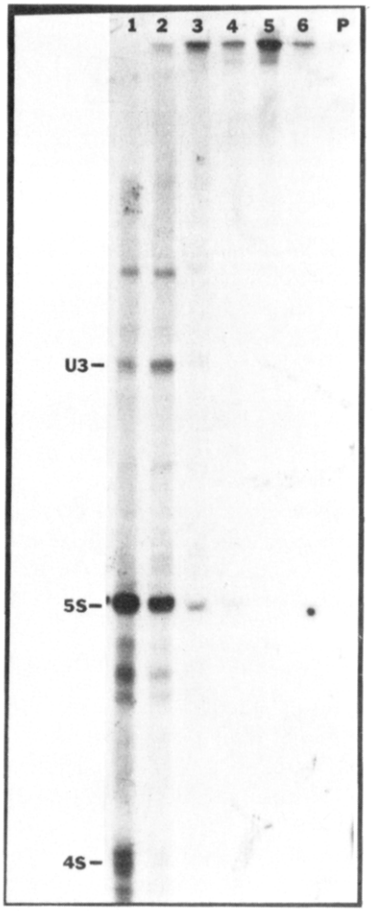

FIGURE 2: Autoradiogram of a polyacrylamide gel showing the sedimentation positions of ^{32}P-labeled small nucleolar RNP particles, when mixed with a Tris extract of unlabeled nucleoli. The ^{32}P-labeled small nucleolar RNP particles were obtained from pool 2 (Figure 1) of a 5–50% sucrose density gradient on which 3 mL of a Tris extract obtained from 100 mg of ^{32}P-labeled nucleoli was centrifuged as described under Experimental Procedures. One milliliter of this sample was then dialyzed against 0.01 M Tris (pH 7.0) and mixed for 60 min at 4 °C with 10 mL of a Tris extract obtained from 300 mg of unlabeled nucleoli. Three milliliters of the mixed sample was then centrifuged on a 5–50% sucrose density gradient as described under Experimental Procedures, and pools 1–6 and the pellet were obtained as shown in Figure 1A.

since it caused streaking and increased background levels of staining. This was accomplished by heating the samples to 63 °C to release small RNAs from high molecular weight RNA and centrifugation on 5–40% sucrose density gradients (Prestayko et al., 1971) or 1 M NaCl elution of low molecular weight RNA from DEAE-Sepharose columns (Ōkada et al., 1976). RNA patterns were identical before and after removal of high molecular weight RNA except for improved resolution and lower background. The RNA bands were visualized by silver staining using the Bio-Rad silver staining kit or by autoradiography. Stained bands were quantitated by densitometry using a Kontes 800 densitometer and a Hewlett Packard 3390A integrator. Radioactive RNA bands were quantitated by excising the band and determining ^{32}P by Cerenkov counting. Equal amounts of gel above and below the band were counted to determine background.

Results

Binding of U3 RNA to Preribosomal RNP Particles. To determine the distribution of U3 RNA in nondenatured extracts of Novikoff hepatoma nucleoli, Tris extracts, which contained 80% of total U3 RNA, were centrifuged on 5–50% sucrose density gradients (Figure 1A). The fractionated gradients were divided into six pools and a pellet. The major high molecular weight RNA components of pools 4 and 5 and the pellet, as determined by sucrose density gradient sedimentation, were 28S and 32S RNA (Prestayko et al., 1972) in the preribosomal RNP particles. The snRNAs of each pool are shown in Figure 1B. 5S, 5.8S, and 7-1 RNAs were present mainly in pools 4 and 5, as was 8S RNA, a putative precursor to 5.8S RNA (Reddy et al., 1983a). Pools 1 and 2 contained about 15% of nucleolar U3 RNA, which sediments at less than 20 S (McEwen 1967). Pools 4, 5, and 6 and the pellet contained 25%, 35%, 2%, and 5% of the U3 RNA, respectively. Accordingly, approximately 65% of U3 RNA was associated with preribosomal RNP particles.

The possibility that U3 RNA might bind nonspecifically to preribosomal RNP particles was examined. An aliquot of pool 2, obtained from a Tris extract of ^{32}P-labeled nucleoli, was mixed with a Tris extract of unlabeled nucleoli and centrifuged on a 5–50% sucrose gradient. No detectable ^{32}P-labeled U3 RNP was recovered from the portion of this gradient containing preribosomal RNP particles (Figure 2), indicating that U3 RNA did not artifactually bind to preribosomal RNP particles.

Table I: Effect of Temperature on the Content of U3, 5.8S, and 5S RNAs in Preribosomal RNP Particle Pellets[a]

RNA	temp (°C)					
	5	25	35	45	55	65
U3	100	110	95	105	50	10
	(1640[b])	(1805)	(1526)	(1740)	(861)	(145)
5.8 S	100	105	100	95	<5	<5
	(3183)	(3376)	(3220)	(3078)	(78)	(63)
5 S	100	105	85	75	15	<5
	(8364)	(8789)	(7298)	(6396)	(1173)	(195)

[a] The values represent the percentage of counts recovered for each RNA relative to counts determined at 5 °C. [b] The values in parentheses are the actual counts above background obtained from the gel shown in Figure 3. Counts were determined as described under Experimental Procedures.

The autoradiogram in Figure 3 shows the small RNAs associated with preribosomal RNP particles after heating to temperatures between 5 and 65 °C. Tris extracts of ^{32}P-labeled nucleoli were incubated for 20 min at 5–65 °C, and then the preribosomal RNP particles were pelleted as described under Experimental Procedures. Counts recovered in the pellet up to 55 °C varied by less than 10%, and counts in the pellet at 65 °C were reduced by 23% (average of two experiments), indicating that even at 65 °C most preribosomal particles remained largely intact.

Table I gives the percentage of each RNA in the preribosomal RNP pellet at each temperature relative to that recovered at 5 °C. The low counts in U3 RNA, relative to counts in 5S and 5.8S RNAs, are due to the more than 6-fold lower specific activity of U3 RNA compared to nucleolar 5S and 5.8S RNAs, as previously reported (Mauritzen et al., 1970). U3 RNP particles were apparently tightly bound to preribosomal RNP particles since a major reduction in bound U3 RNP was not apparent up to 45 °C. At 55 °C, 50% of the U3 RNP remained associated with preribosomal RNP particles while less than 5% of 5.8S RNA and only 14% of 5S RNA remained.

Binding of U3 RNA to Deproteinized Preribosomal RNA. Preribosomal RNP particle enriched pools 4 and 5 from the sucrose gradient shown in Figure 1 were deproteinized at 22 °C as described under Experimental Procedures. The deproteinized fractions were placed on 5–40% sucrose density gradients. The small RNAs from the 4–8S region (lane 1), the 8–18S region (lane 2), and the 28S and larger region (lane 3) are shown in Figure 4A. Of the U3 RNA from pools 4 and 5, 15% (equivalent to about 10% of total nucleolar U3 RNA) remained associated with 28S and larger RNA (Table II). Only 0.4% of the proteins originally present in pools 4 and 5 was left in this region of the gradient. Similar results were obtained when deproteinized total nucleolar RNA or deproteinized RNA from the pellet of the Tris gradient was centrifuged on 5–40% sucrose density gradients.The results with total nucleolar RNA are shown in Figure 4B. Loss of 5S RNA from 28S and larger RNA was essentially complete.

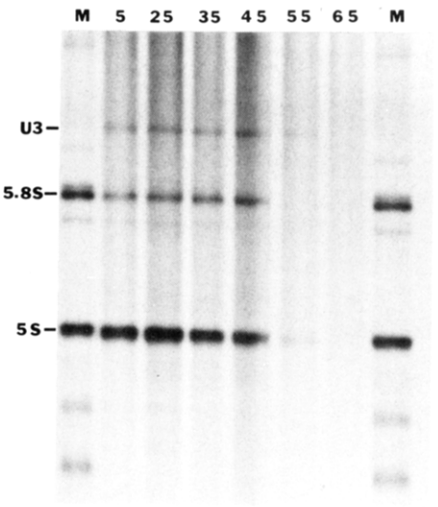

FIGURE 3: Autoradiograph of a polyacrylamide gel showing the small nucleolar RNAs associated with preribosomal RNP particles after a 15-min incubation at 5, 25, 35, 45, 55, and 65 °C. Tris extracts of ^{32}P-labeled Novikoff hepatoma nucleoli were incubated at the temperatures indicated and centrifuged at 87000g_{av} for 2 h in a Ti 50 rotor. RNA from the pellet is shown.

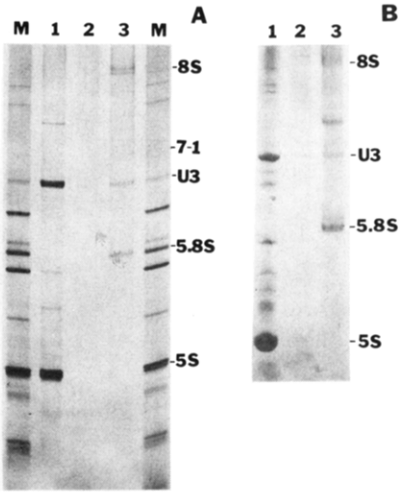

FIGURE 4: Silver-stained gels of the small nucleolar RNAs from the 4–8S (lane 1), 8–18S (lane 2), and 28S and larger (lane 3) regions of sucrose gradients on which 22 °C extracted RNA from pools 4 and 5 (panel A) or total nucleolar RNA (panel B) was applied. Lane M contains 4–8S nuclear RNA.

The binding of U3 RNA to 22 °C deproteinized preribosomal RNA was examined by repeating the deproteinization procedure (Figure 5A), by incubation with urea (Figure 5B), and by heating (Figure 5C) prior to centrifugation. Repeated deproteinization (Figure 5A) reduced the amount of bound U3 by les than 5%, which indicates that the bound U3 RNA fraction is base paired to preribosomal RNA (Prestayko et al., 1971). The extraction with phenol and prolonged incu-

Table II: Association States of U3, 5.8S, and 5S RNAs in 0.01 M Tris Extracts of Novikoff Hepatoma Nucleoli

RNA	% extracted[a]	% in soluble pool[b]	% bound to preribosomal RNP particles[c]	% bound to preribosomal RNP particles by protein[d]	% base paired to preribosomal RNA[e]
U3	80	15	65	55	10
5.8 S	90	<5	>90	0	>90
5 S	85	5	75	75	0

[a] Percentage of each nucleolar RNA (extracted plus unextracted) in the Tris extract (Figure 1B, lanes R and T). [b] Percentage of each RNA in pools 1 and 2 (Figure 1B, lanes 1 and 2). [c] Percentage of each RNA in pools 4–6 and the pellet (Figure 1B, lanes 4–6 and pellet). [d] Percentage of each RNA released from preribosomal RNP particles (Figure 3A, lanes 1 and 2) by deproteinization. [e] Percentage of each RNA bound to preribosomal RNA after deproteinization (Figure 2A, lane 3).

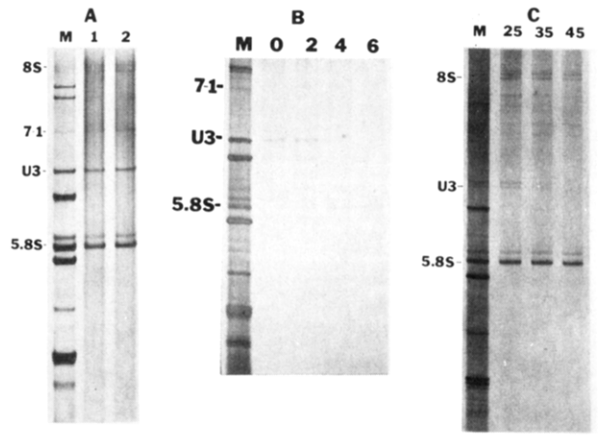

FIGURE 5: Silver-stained gels of the small nucleolar RNAs in the 28S and larger region of sucrose gradients after various treatments. (A) Lanes 1 and 2 show the small RNAs associated with 28S and larger RNA after one and two (respectively) cycles of deproteinization of total nucleolar RNA with phenol/SDS and proteinase K at 22 °C as described under Experimental Procedures. (B) Small nucleolar RNAs associated with 28S and larger RNA after incubation of nucleolar RNA with 0, 2, 4, and 6 M urea in 100 mM NaCl and 10 mM Tris (pH 7.0) for 20 min at 22 °C. (C) Small nucleolar RNAs associated with 28S and larger RNA after heating 28S and larger RNA to 25, 35, and 45 °C for 20 min in 100 mM NaCl and 10 mM Tris (pH 7.0). Lane M contains 4–8S nuclear RNA.

bation with SDS during proteinase K digestion did not significantly decrease the amount of base-paired U3 RNA. U3 RNA was less strongly bound than 5.8S RNA since U3 RNA was dissociated at 4 M urea (Figure 5B), and 5.8S RNA was not released with 6 M urea. Heating to 35 °C dissociated 50% of the base-paired U3 RNA, and heating to 45 °C completely dissociated the bound U3 RNA while 5.8S RNA was not released at 45 °C.

Discussion

The present results, summarized in Table I, suggest the existence of at least three pools of nucleolar U3 RNP: (a) a free, soluble, nonsedimenting pool; (b) a pool bound to preribosomes by protein–protein or protein–RNA interactions; and (c) a pool base paired to preribosomal RNA, which may be stabilized by proteins. The free pool contained 15% of U3 RNA. The largest pool, which contained 55% of the U3 RNA, was bound to preribosomal RNP particles by protein–protein or protein–RNA interactions. Only 10% of U3 RNA was base paired to preribosomal RNA.

Twenty precent of nucleolar U3 RNP was not solubilized by the 0.01 M Tris extraction procedure. Digestion with DNase also did not release this fraction (unpublished results). Several reports (Miller et al., 1978; Ciejek et al., 1982; Vogelstein & Hunt, 1982) have shown that a fraction of the U-snRNAs is associated with nuclear matrix. The 0.01 M Tris insoluble fraction of U3 RNP may be bound to a nucleolar matrix.

The base-paired pool of U3 RNA appears to be weakly bound to preribosomal RNA, since heating to 35 °C dissociates 50% and 4 M urea completely dissociates the base-paired U3 RNA. Recently proposed models for the base pairing of U3 RNA (Crouch et al., 1983; Bachellerie et al., 1983) and 5.8S RNA (Michot et al., 1982; Walker & Pace, 1983) to different regions of ribosomal RNA suggest that 5.8S RNA is bound by more extensive regions of sequence complementarity than is U3 RNA. Consistent with this, U3 RNA was more easily dissociated by heat or urea than 5.8S RNA.

In contrast to the relatively weak base pairing of U3 RNA to preribosomal RNA found in deproteinized RNA, bonds

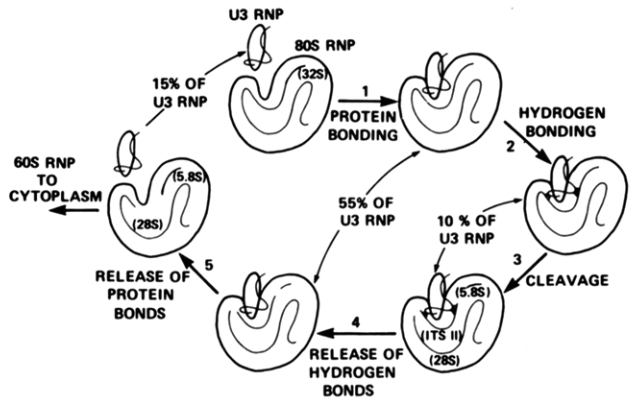

FIGURE 6: Model for the binding and release of U3 RNP particles from preribosomal RNP particles. Step 1, free U3 RNP particles bind to 80S RNP particles through protein bonds; step 2, U3 RNA of the bound U3 RNP base pairs with 32S preribosomal RNA (Prestayko et al., 1971; Crouch et al., 1983; Bachellerie et al., 1983); step 3, 32S preribosomal RNA is processed to 28S and 5.8S RNAs; step 4, base pairing between U3 RNA and preribosomal RNA is released; step 5, protein bonds between U3 RNP and preribosomal RNP particles are released and the free RNP particles return to the soluble pool of U3 RNP particles. The percentages of U3 RNP particles shown for the different states of association with preribosomal RNP particles are taken from Table II. The 65% of extractable U3 RNP bound to preribosomal RNP particles (Table II) consists of the 10% of U3 RNP which is both base paired and protein bound between steps 2 and 4 and the 55% which is bound only by protein before step 2 and after step 3. The 20% of U3 RNP which was not extracted with 0.01 M Tris is not shown since it could not be analyzed for binding to preribosomal RNP particles.

between preribosomal RNP particles and U3 RNP were more stable with respect to temperature than the bonds to 5S or 5.8S RNAs. The tighter binding in the presence of protein probably reflects the strength of the protein-dependent bonds between U3 RNP and preribosomal RNP particles. Proteins of U3 RNP or preribosomal RNP particles may stabilize the base pairing of U3 RNA to preribosomal RNA, since 50% of the base-paired fraction of U3 RNA was found to be unstable at 35 °C.

Since U3 RNP appears to be confined to the nucleolus and preribosomal RNP particles are continuously transported from the nucleolus, it is evident that U3 RNP must go through an association–dissociation cycle with respect to preribosomal RNP particles. A possible model for the binding and release of U3 RNP from preribosomal RNP particles is shown in Figure 6. This model is consistent with the three states of U3 RNP identified in this study, the presence of U3 RNP particles in both 60S and 80S preribosomal RNP particles (pools 4 and 5; Figure 1), and earlier proposals (Crouch et al., 1983; Bachellerie et al., 1983) that base-paired U3 RNA is involved in the processing of 32S preribosomal RNA to 28S and 5.8S RNAs. Since 80S preribosomal RNP particles contain 32S RNA (Auger-Buenida et al., 1978), the proposed substrate for U3 RNP processing (Crouch et al., 1983; Bachellerie et al., 1983), the initial binding of U3 RNP may occur at this step or some earlier step of preribosome formation. As shown in step 1, free U3 RNP may bind to 80S preribosomal RNP particles in close proximity to processing sites of 32S preribosomal RNA. The initial association may require protein binding which has been previously observed for the binding of U1 RNP to hnRNA (Mount et al., 1983). The short (Lerner et al., 1980; Crouch et al., 1983; Bachellerie et al., 1983) and weak (Figure 5B,C) base pairing between small RNAs and large precursor RNAs may require protein binding to increase the probability of binding and to ensure specificity of binding. In step 2, the close proximity of complementary

sequences in 32S RNA and U3 RNA promotes the formation of the base-paired form of U3 RNA (see Figure 3A,B; Prestayko et al., 1971; Zieve & Penman, 1976; Calvet & Pederson, 1981). The stability of these short hybrids may depend on the initial protein bonds formed between 80S RNP particles and U3 RNP in step 1, since in the absence of protein they begin to melt at 35 °C (Figure 5C). Two regions of hybridization between U3 RNA and 32S RNA are shown, consistent with the two regions of binding proposed by Bachellerie et al. (1983). In step 3, 32S RNA is processed to 28S and 5.8S RNAs. This processing probably requires the presence of base-paired U3 RNA (Crouch et al., 1983; Bachellerie et al., 1983). In step 4, the weak hybrids between U3 RNA and preribosomal RNA are released. The release of base pairing prior to the release of protein bonds is suggested by the presence of some U3 RNP in the 60S preribosomal RNP peak which is apparently bound only by protein bonds. U3 RNP is returned to the free pool by release of protein bonds between U3 RNP and the 60S RNP particle (step 5) prior to the transport of the 60S RNP particles from the nucleolus. Preliminary data indicate that a portion of the U3 RNP which could not be extracted with 0.01 M Tris is also base paired to preribosomal RNA. This nonextractable pool of U3 RNP may also be bound to preribosomal RNP particles, perhaps at a matrix-associated step.

At this time, determination of the binding and processing site(s) of U3 RNP on 32S RNA and preribosomal RNP particles is dependent on sequence comparisons (Bachellerie et al., 1983) which show evolutionary conservation of complementary sequences in U3 RNA and 32S RNA near processing sites. Efforts are under way to obtain pure U3 RNP in order to directly verify the proposed binding sites and processing events.

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References

Auger-Buenida, M., Hamelin, R., & Tavitian, A. (1978) *Biochim. Biophys. Acta 521*, 241–250.

Bachellerie, J.-P., Michot, B., & Raynal, F. (1983) *Mol. Biol. Rep. 9*, 79–86.

Busch, H., Reddy, R., Rothblum, L., & Choi, Y. C. (1982) *Annu. Rev. Biochem. 51*, 617–654.

Calvet, J. P., & Pederson, T. (1981) *Cell (Cambridge, Mass.) 26*, 363–370.

Ciejek, E. M., Nordstrom, J. L., Tsai, M.-J., & O'Malley, B. W. (1982) *Biochemistry 21*, 4945–4953.

Crouch, R. J., Kanaya, S., & Earl, P. L. (1983) *Mol. Biol. Rep. 9*, 75–78.

Frederiksen, S., & Hellung-Larsen, P. (1974) *FEBS Proc. 33*, 175–180.

Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L., & Steitz, J. A. (1980) *Nature (London) 283*, 220–224.

Matsui, S., Fuke, M., & Busch, H. (1977) *Biochemistry 16*, 39–45.

Mauritzen, C. M., Choi, Y. C., & Busch, H. (1970) *Methods Cancer Res. 6*, 253–282.

McEwen, C. R. (1967) *Anal. Biochem. 20*, 114–149.

Michot, B., Bachellerie, J.-P., & Raynal, F. (1982) *Nucleic Acids Res. 10*, 5273–5283.

Miller, T. E., Huang, C.-Y., & Pogo, A. O. (1978) *J. Cell Biol. 76*, 692–704.

Mount, S., Petterson, I., Hinterberger, M., Karmas, A., & Steitz, J. (1983) *Cell (Cambridge, Mass.) 33*, 509–518.

Okada, N., Harada, F., & Nishimura, S. (1976) *Nucleic Acids Res. 3*, 2593–2603.

Prestayko, A. W., Tonato, M., & Busch, H. (1971) *J. Mol. Biol. 47*, 505–515.

Prestayko, A. W., Tonato, M., & Busch, H. (1972) *Biochim. Biophys. Acta 269*, 90–103.

Reddy, R., & Busch, H. (1981) *Cell Nucl. 7*, 261–306.

Reddy, R., Li, W., Henning, D., Choi, Y. A., Nohga, K., & Busch, H. (1981) *J. Biol. Chem. 256*, 8452–8457.

Reddy, R., Rothblum, L. I., Subrahmanyam, C. S., Liu, M. H., Henning, D., Cassidy, B., & Busch, H. (1983a) *J. Biol. Chem. 258*, 584–589.

Reddy, R., Spector, D., Henning, D., Liu, M., & Busch, H. (1983b) *J. Biol. Chem. 258*, 13965–13969.

Ro-Choi, T. S., Raj, B. K., Pike, L. M., & Busch, H. (1976) *Biochemistry 15*, 3823–3828.

Vogelstein, B., & Hunt, B. F. (1982) *Biochem. Biophys. Res. Commun. 105*, 1224–1232.

Walker, T. A., & Pace, N. R. (1983) *Cell (Cambridge, Mass.) 33*, 320–322.

Wise, J. A., & Weiner, A. M. (1980) *Cell (Cambridge, Mass.) 22*, 109–118.

Zieve, G., & Penman, S. (1976) *Cell (Cambridge, Mass.) 8*, 19–31.