Identification of a U8 snoRNA-specific Binding Protein*

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Eukaryotic nucleoli contain a large and diverse population of small nucleolar ribonucleoprotein particles (snoRNPs) that play diverse and essential roles in ribosome biogenesis. We previously demonstrated that U8 snoRNP is essential for processing of both 5.8 and 28 S rRNA. The RNA component of the U8 RNP particle is necessary but not sufficient for processing. Using an electrophoretic mobility shift assay, we enriched for U8-specific binding proteins from Xenopus ovary extracts. UV cross-linking reactions with partially purified fractions implicated a 29-kDa protein directly binding to U8 RNA. This protein interacted specifically and with high affinity with U8 snoRNA; it did not bind other snoRNAs and is probably not a common component of all snoRNPs. This is the first report of a protein component specific to an snoRNP essential for processing of the large ribosomal subunit in vertebrates.

The small nucleolar ribonucleoprotein particles (snoRNPs)¹ are known to play various roles at several different steps along the complex process of ribosome biogenesis, including directing site-specific methylation, pseudouridylation, and processing of the rRNA precursors (reviewed in Refs. 1–3). U8 snoRNP is functionally unique. It is the only known snoRNP that has been shown to be essential for processing of both 5.8 and 28 S rRNAs. These two RNAs, together with 5 S RNA, make up the RNA components of the large ribosomal subunit. Conversely, in yeast and vertebrates, a total of six snoRNAs have been shown to affect processing of the 18 S rRNA, the only rRNA present in yeast, whereas just a handful are required for accurate cleavage of pre-rRNA, the small ribosomal subunit. To date, no snoRNA other than U8 and no additional trans-acting cleavage or accessory proteins have been identified that are required in vertebrates for processing of both 5.8 and 28 S rRNAs.

The nucleolus contains a large number of snoRNPs. Over 100 different species of snoRNAs have been identified in yeast (Saccharomyces cerevisiae) and vertebrates (reviewed in Refs. 1–3). These snoRNAs can be placed in two groups based on function. The vast majority of the snoRNAs are involved in directing site-specific posttranscriptional modification of the rRNA, whereas just a handful are required for accurate cleavage of pre-rRNA. Within each functional group, the snoRNAs can be subdivided into two classes, based on common sequence elements and shared proteins; those of the C and D box class and those including the H/ACA sequences (reviewed in Refs. 4 and 5). snoRNAs containing the H/ACA sequences associate with the proteins fibrillarin (called NOP1p in yeast), Nop56p, and Nop55/58p (Refs. 6 and 7 and reviewed in Ref. 1). The snoRNAs containing the H/ACA box sequences associate with the yeast proteins Gar1, Chf5p, Nhp2p, and Nop10p (8–12). Most of these data have been obtained in yeast, and at the level of molecular biology, there is generally less information available regarding rRNA processing in vertebrates.

In many cellular RNPs (for example, the small nuclear ribonucleoprotein particle, telomerase, and the snoRNPs) the RNA component within the particle directs the precise site or alignment with the target template, whereas the protein components of the particle provide structural integrity, specificity, and/or catalytic activity (reviewed in Refs. 4, 5, 13, and 14). Whereas U8 is a C/D box snoRNA and presumably associates with the proteins described above, there must be trans-acting factors or U8-specific proteins that provide the unique in vivo function of U8 RNA. Thus, we are examining the protein subunits of U8 RNP to identify the protein constituents and gain a better understanding of the molecular mechanisms by which U8 facilitates pre-rRNA processing.

Here, we identify a protein that binds U8 snoRNA with high specificity. Conventional biochemical methods were used to enrich for U8-binding activity as measured in vitro. This novel 29-kDa protein was shown to bind U8 RNA specifically; it was efficiently cross-linked to U8 RNA, and neither associated with nor was its binding to U8 competed by other C and D box snoRNAs. This protein represents the first candidate for an integral component of U8 snoRNP, an essential nucleolar complex responsible for accumulation of mature large ribosomal subunits in vertebrates.

EXPERIMENTAL PROCEDURES
Preparation of Xenopus Ovary Extract

Whole ovary was surgically removed from mature female frogs (obtained from Xenopus I; Dexter, MI) and minced. All subsequent steps were performed on ice. The minced tissue was washed ten times with ice-cold PBS buffer and allowed to settle for 3 min after each wash. After the final wash, the tube was centrifuged briefly at 1500 × g, and the volume of packed ovary was determined. The ovary was then washed once with three volumes of cold wash buffer (20 mM KCl, 0.5 mM EDTA, 5 mM Tris-HCl (pH 7.4), 0.05 mM spermine, 0.12 mM spermidine, 200 mg/ml aprotinin, 0.2 mM phenylmethylsulfonyl fluoride) and centrifuged again. Ovary was resuspended in one volume of break buffer (200 mM KCl, 1 mM EDTA, 10 mM Tris-HCl (pH 7.4), 0.1 mM spermine, 0.24 mM spermidine, 2 μM aprotinin, 2 mM phenylmethylsulfonyl fluoride, and 1% triethylglycol) and transferred to a Dounce homogenizer (model 357546, Wheaton). Manual homogenization was performed by 10 strokes with the loose pestle followed by 3 strokes with the tight-fitting pestle. The resulting suspension was centrifuged at 13,000 × g for 25 min at 4 °C. The cleared supernatant, typically 20–30 mg/ml total protein, was either used immediately or was stored at −80 °C with the addition of 30% glycerol (w/v; final concentration).

Protein Purification

Ammonium Sulfate—The cleared ovary extract was brought to 30% saturation with solid ammonium sulfate and mixed gently at 4 °C for 3 h. The resulting slurry was centrifuged for 20 min at 12,000 × g. The

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1 The abbreviations used are: snoRNP, small nucleolar ribonucleoprotein particle; PAGE, polyacrylamide gel electrophoresis.
30% pellet was saved, and the resulting supernatant was then brought to 40% saturation with ammonium sulfate, then 50% and then 80% saturation in turn. The four ammonium sulfate pellets were resuspended in 0.1 M starting volume of ovary extract with Buffer I (20 mM Tris-HCl (pH 7.6), 1 mM EDTA, 1 mM dithiothreitol and 2% (v/v) glycerol). The salt in the sample was decreased using a desalting column (model 43243, Pierce) that was previously equilibrated in Buffer I plus 50 mM NaCl.

DEAE-Sepharose—The 50–80% ammonium sulfate pellet fraction was loaded onto a DEAE-Sepharose CL-6B (Amersham Pharmacia Biotech) column (7 ml) previously equilibrated with Buffer I plus 60 mM NaCl. The column was washed with the same buffer until the A_{260} reached baseline (a minimum of 10 column volumes). The bound proteins were eluted with a 5-column volume linear gradient (60–300 mM NaCl) in Buffer I. Fractions that contained U8-binding activity as identified by gel shift analysis (see below) were pooled and used for further purification.

Heparin-Sepharose—Fractions containing peak U8-binding activity from the DEAE-Sepharose column were pooled then diluted with one volume of Buffer I and loaded onto a heparin-Sepharose CL-6B column (3 ml) equilibrated with Buffer I plus 100 mM NaCl. The column was washed with the same buffer until the A_{260} reached baseline (typically 5 column volumes). Bound proteins were eluted with a 10-column volume linear salt gradient (100–300 mM NaCl) in Buffer I. One-half ml fractions were collected and assayed for U8-binding activity using the gel shift assay as described below. Fractions that contained the highest U8-binding activity were examined and pooled for use in further purification.

Superdex S-75 SMART Column—Fractions containing peak U8-binding activity were pooled and concentrated to 50 µl using Centriprep 10 concentrators (Amicon) and applied to a Superdex S-75 column connected to a SMART chromatography system (Amersham Pharmacia Biotech). The column was equilibrated in Buffer I plus 100 mM NaCl. The retention times/volumes of proteins were monitored by following the A_{280} and processed with the software SMART MANAGER supplied by Pharmacia. Fractions were collected and analyzed for both U8-binding activity by gel shift analysis and protein complexity by SDS-polyacrylamide gel electrophoresis (PAGE).

Deoxycholate-Trichloroacetic Acid Precipitation of Proteins for SDS-PAGE Analysis

Quantitative protein precipitation was performed essentially as per the method of Bensadoun and Weinstein (15), with slight modifications. When the concentration of proteins was lower than 10 µg/ml sodium deoxycholate was added to a final concentration of 50 µg/ml. After 30 min on ice, cold trichloroacetic acid (Sigma) was added to a final concentration of 7% (v/v). The mixture was incubated on ice for 1 h and then centrifuged at 4 °C for 20 min at 16,000 × g in a microcentrifuge. The pellets were dissolved in SDS Laemmli buffer (16) and boiled for 5 min prior to analysis by SDS-PAGE analysis.

Gel Shift Analysis

RNA binding assays were performed as described in Ref. 17 with the following modifications. Typically, binding reactions (5–14 µl) containing buffer (pH 7.6, 5 mM Tris-HCl, 2.5 mM spermidine, 0.5 mM dithiothreitol, 0.2 mM EDTA, 10% glycerol (v/v), and 50 mM NaCl), yeast tRNA (109495, Roche Molecular Biochemicals) (0.3–1.2 µg, corresponding to 200–800-fold molar excess), an aliquot of protein fraction (1–10 µl), and 60 fmol of 32P-Radiolabeled RNA. The mixture was incubated for 16 min at room temperature then loaded onto a 4% native polyacrylamide gel (66:1 acrylamide:bis) containing 0.6 M Tris-HCl (pH 7.6), 1 mM EDTA, 1 mM dithiothreitol and 2% (v/v) glycerol. The gel (14 × 14 × 0.75 cm) was run for 365 nm at 280 and processed with the software SMART MANAGER supplied as a substrate for RNP particle formation. In order to identify and characterize the proteins that bind U8 snoRNA and are involved in formation of U8 particles, we established conditions for a gel shift analysis that would serve as a functional assay for the presence of U8-specific binding proteins.

Extracts made from Xenopus ovary were examined for the presence of proteins capable of binding U8 RNA. Binding reactions containing crude ovary extract, 32P-labeled Xenopus U8 snoRNA, and nonspecific rRNA competitor were used to identify conditions for specific U8 complex formation in vitro. Fig. 1 shows a gel shift assay with crude Xenopus ovary extract and 3 ng of 32P-labeled U8 RNA (Fig. 1, lane 2). Essentially all of the labeled RNA present in this reaction was shifted to a mobility consistent with an RNP complex, indicating reactions assemblings comprising a particles present in crude ovary extract, and no bound RNA was present (Fig. 1, compare lanes 1 and 2). The addition of increasing amounts of unlabeled U8 RNA (lanes 3–6) or nonspecific 5 S RNA (lanes 8–11) competitor RNA at 15-, 30-, 60-, or 120-fold molar excess prevented the formation of some but not all of the complexes. This indicated that some of the observed complexes represented nonspecific interactions, whereas others were due to the formation of more specific U8-containing complexes.

RESULTS

U8 snoRNP Complex Formation In Vitro

We previously reported that in vitro synthesized U8 RNA injected into Xenopus oocytes will form RNP particles that localize to the nucleus and function in pre-rRNA processing (18, 19). These data demonstrated that exogenous U8 RNA serves as a substrate for RNP particle formation. In order to identify and characterize the proteins that bind U8 snoRNA and are involved in formation of U8 particles, we established conditions for a gel shift analysis that would serve as a functional assay for the presence of U8-specific binding proteins.

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![RNA Transcription and UV Cross-linking](image)

![U8 snoRNA-binding Protein](image)
identification of complexes sensitive to competition with U8 RNA but not with 5 S RNA suggests that this assay provides a means of measuring specific binding to U8 RNA.

**Enrichment for U8-binding Protein**

The complexity of the band pattern observed in the gel shift analysis indicated that U8 RNA may be capable of forming complexes with several different protein factors. To identify specific U8-binding proteins, we focused on pooled fractions displaying U8-binding activity, as determined by gel shift analysis, and followed the binding activity through several fractionation steps. Thus, we generated a fractionation protocol that resulted in the identification of U8-specific binding protein of 29 kDa. This enrichment protocol is summarized in Fig. 2 and can be outlined as follows.

**Ammonium Sulfate Precipitation**—Crude Xenopus ovary extract was fractionated by differential precipitation with ammonium sulfate. The desalted 0–30, 30–40, 40–50, and 50–80% ammonium sulfate pellets were assayed for U8 binding activity via gel shift analysis. Fig. 3 shows the binding activity present in each of these fractions. Lanes 2–6 each contain 10 μg of protein; however, the ability to shift a constant amount (3 ng) of U8 RNA differed dramatically for each fraction. These results indicated that proteins capable of interacting with U8 RNA were present in each of the ammonium sulfate fractions, but the identity and the abundance of these proteins presumably differed in each fraction.

To determine which of the ammonium sulfate fractions should be the focus of additional purification, each of the four fractions was examined for specificity of U8 binding. Competition binding assays were performed with protein from each ammonium sulfate fraction in the presence of specific (U8) and nonspecific (tRNA and 5 S RNA) competitors (data not shown). These competition studies demonstrated that proteins in the 50–80% ammonium sulfate fraction appeared to be binding U8 RNA with high specificity generating at least two major complexes (designated complexes L and U in Fig. 3, lane 6).

**DEAE-Sepharose Column**—To further enrich for U8-binding activity in the 50–80% ammonium sulfate fraction, the proteins in this fraction were loaded onto a DEAE-Sepharose column. Under the conditions used, most of the proteins flowed through the DEAE column, but the detectable U8-binding activity was present in the elution profile. The proteins/binding activities that generated the two U8 RNA complexes (Fig. 3, lane 6, U and L) could be separated from each other by elution with a linear salt gradient (data not shown, also see Fig. 4 and under “Experimental Procedures”). Fractions eluted from the DEAE column between approximately 160–200 mM NaCl contained proteins that generated complexes that comigrated in a native gel with the complex L seen in the 50–80% AS fraction. Complex L was not readily detectable in the crude extract (Fig. 1) but was clearly visible in the 50–80% ammonium sulfate fraction (Fig. 3, lane 6).

The DEAE fractions containing U8 binding activities resulting in complex L formation (eluted at approximately 160–200 mM NaCl) were pooled separately from those forming complex U (eluted at approximately 210–300 mM NaCl). Each pool was examined separately for specificity by measuring the effects of tRNA competition on U8 binding, shown in Fig. 4. The L complex was found to be more resistant to tRNA competitor, consistent with greater specificity in binding (compare Fig. 4,
The specificity of U8 complex formation was examined by gel shift analysis. Increasing amounts of tRNA competitor (i.e., the molar ratio of tRNA to labeled U8 RNA) is shown at the top. Lane 1 is U8 RNA with no added protein. Lanes 2–6 contain pooled protein fractions elution from the DEAE column between 210 and 300 mM NaCl. Lanes 7–11 contain pooled fractions eluting from the DEAE column between 160 and 200 mM NaCl. The mobilities of complexes designated U and L are indicated at the sides.

lanes 2–6 and 7–11). Additional competition binding assays were performed using specific (sense strand U8) or nonspecific (S S) RNAs to further determine the specificity of U8 binding (data not shown). These data further supported the conclusion that the L complex demonstrated higher specificity for U8 binding. Thus, we chose to pursue the protein(s) responsible for L complex formation.

**Heparin-Sepharose Column**—As a next step in the enrichment of U8-binding proteins, those fractions from the DEAE column that contained maximal L complex formation activity were pooled. This pool was loaded onto a heparin-Sepharose column. The U8-binding activity was retained on the column, and bound proteins were eluted with a linear gradient of 100–300 mM NaCl (Fig. 5A). Fractions eluted from this column were analyzed by gel shift analysis for the ability to bind U8 RNA (Fig. 5B). The gel shift assay clearly demonstrated only one peak of U8-binding activity, which resulted in a shifted band that comigrated with complex L (Fig. 5B, fractions 29–35). Those fractions with peak U8-binding activity were pooled, and an aliquot of pooled material was examined by SDS-PAGE. Examination of the protein composition indicated that although the pattern was less complex than previous steps, a number of major protein bands were detected by silver staining (data not shown, but refer to Fig. 8, lane 4).

To correlate U8 binding activity with one or just a few bands, the extent of binding activity in each fraction across a heparin-Sepharose profile was determined by gel shift assay. Proteins in the corresponding fraction were examined by SDS-PAGE, and a portion of this gel is shown in Fig. 5C. The binding activity as measured by gel shift assay (shown above Fig. 5C) best corresponded to the elution profile of a 29-kDa band detected by SDS-PAGE.

**UV Cross-linking Proteins to U8 snoRNA**

To test whether the 29-kDa protein present in the heparin-Sepharose fraction was responsible for the U8 binding activity in vitro, UV cross-linking experiments were performed. U8 RNA was transcribed in the presence of [32P]-labeled RNA and 4-thiouridine, a photoactivatable nucleotide analogue. To examine whether the presence of 4-thiouridine affected the mobility of complex formation involving the U8 RNA, UV cross-linking experiments were performed. U8 RNA was transcribed in the presence of [32P]-labeled RNA and 4-thiouridine, a photoactivatable nucleotide analogue. To examine whether the presence of 4-thiouridine affected the mobility efficiency or specificity of complex formation involving the U8 RNA, UV cross-linking experiments were performed. U8 RNA was transcribed in the presence of [32P]-labeled RNA and 4-thiouridine, a photoactivatable nucleotide analogue. 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**Fig. 4. Examining specificity of complexes U and L by tRNA titration.** The specificity of U8 complex formation was examined by gel shift analysis. Increasing amounts of tRNA competitor (i.e., the molar ratio of tRNA to labeled U8 RNA) is shown at the top. Lane 1 is U8 RNA with no added protein. Lanes 2–6 contain pooled protein fractions elution from the DEAE column between 210 and 300 mM NaCl. Lanes 7–11 contain pooled fractions eluting from the DEAE column between 160 and 200 mM NaCl. The mobilities of complexes designated U and L are indicated at the sides.

**Fig. 5. Enrichment for U8 RNA binding activity using a heparin-Sepharose column.** A, DEAE-Sepharose fractions with the highest U8 RNA binding activity were pooled and loaded on a heparin-Sepharose column. Proteins were eluted with a linear gradient of 100–300 mM NaCl (indicated by the dotted line). The elution profile of protein, as determined by A280, is indicated by the solid line. The binding activity resulting in formation of complex L was assayed by gel shift reactions (seen in B). Amount of complex L formation was determined by quantitatively with a Fuji phosphorimager and the results are shown in arbitrary units (dotted-dashed line). B, the presence of U8 RNA-specific binding proteins was determined by gel shift assay. Ten-μl aliquots from each of the fractions indicated at the bottom were incubated with 32P-labeled U8 RNA and cold tRNA. Free U8 RNA and complex L are indicated. C, to determine which protein could be attributed with U8-binding activity, individual fractions eluted from a heparin-Sepharose column by a linear gradient were analyzed for protein composition by SDS-PAGE and by gel shift assay (data not shown). The binding activity was quantified on a Fuji phosphorimager and plotted in arbitrary units (●) at the top. Shown is the 20–40-kDa region of the SDS-PAGE gel (13% gel). Lane 1 contains proteins from the flow-through fraction. Lanes 2–6 contain consecutive fractions from the regions of the elution gradient previously determined to contain peak U8-binding activity. Lane 7 contains molecular mass markers.
transcribed in vitro

A, cross-linking was observed with this RNA (Fig. 6, subjected to identical cross-linking conditions. No specific RNA-binding protein.

Lane 7 contains 14C-labeled molecular mass markers. The arrow shows the position of a cross-linked protein.

Lane 3 contains protein and labeled RNA probe without competitor. Lanes 3 and 4 contain U8 RNA and protein, plus a 60-fold molar excess of unlabeled U8 RNA or 5 S RNA competitors, respectively. Lane 7 contains 14C-labeled molecular mass markers. The arrow shows the position of a cross-linked protein.

Identifying a Putative U8-binding Protein

To examine 1) whether the U8 binding activity could be directly assigned to the 29-kDa protein identified from the heparin-Sepharose elution profile (see Fig. 5); and 2) whether the U8 binding activity required the presence of additional proteins in this fraction, an analytical scale Superdex S-75 gel filtration column was used to further resolve the proteins in this fraction.

The active U8-binding fractions from a heparin-Sepharose column were pooled and concentrated and then loaded on a Superdex S-75 column on a Amersham Pharmacia Biotech SMART system. Each fraction eluted from the column was analyzed for the ability to bind U8 RNA by gel shift assay (Fig. 7A) and examined for protein content by SDS-PAGE (Fig. 7B). Most of the U8-binding activity was eluted in two consecutive fractions (Fig. 7B, lanes 6 and 7) with trace amounts of this binding activity eluting in the following two fractions (lanes 8 and 9). The profile of the binding activity closely correlates with the elution profile of the 29-kDa protein and does not correlate well with any other visible bands on the gel.

Cross-linking to 4-thiouridine U8 was then used to examine which of the proteins present in the Superdex S-75 column fraction corresponding to lane 7 in Fig. 7B was responsible for U8 binding activity. Cross-linking was performed as described above (and see under “Experimental Procedures”). The resulting pattern of cross-linking was identical to that observed for the heparin-Sepharose pool (Fig. 5); a single protein band of approximately 30 kDa was labeled (data not shown). Thus, these data strongly implicate the 29-kDa protein in direct binding to U8 RNA.

Enrichment of a Specific U8-binding Protein

To examine the enrichment for the U8-binding protein identified here, protein from each of the purification steps was examined by SDS-PAGE. Fig. 8 shows the protein complexity of each step. The final step of purification, containing protein from the Superdex S-75 column fraction corresponding to Fig. 7B, lane 7 above, contains one major protein species and only very small amounts of other polypeptides (Fig. 8, lane 5). We call this 29-kDa Xenopus protein X29.

Characterization of the Binding Specificity of X29

In order to examine the template specificity of the X29 protein, pooled fractions from the heparin-Sepharose column were used for gel shift assays using different snoRNAs as templates. Because U8 is a member of the subclass of snoRNAs containing common sequence elements, the C and D box, it is possible that other snoRNAs also bind X29. Gel shift assays were performed using 32P-labeled U3 and U14 snoRNAs and proteins in the pooled heparin-Sepharose fractions. No specific binding to either U3 or U14 was detected (data not shown). Thus, the X29 protein has a much higher affinity for U8 than for other C and D box snoRNAs.

To provide further evidence for U8 specificity, the ability of trace-labeled U3 and U14 snoRNAs to compete for U8-binding was examined. Fig. 9 shows the results from some of these competition experiments. Labeled U8 RNA (Fig. 9, lane 1) and the shifted complex L (lane 2) are indicated. The addition of
The identification of RNP proteins can help confirm putative activities and may lead to insight about additional roles of U8 RNA in the binding reaction had no detectable effect on the formation of complex L. Likewise, U14 snoRNA present as cold competitor (lanes 7 and 8) failed to compete effectively for binding. These data provide additional evidence that the X29 protein had a much higher affinity for U8 than for other C and D box snoRNAs. Thus, the X29 protein identified in Xenopus ovary extracts is unlikely to be common to all C and D box snoRNPs and may be specific to the U8 RNP alone.

**DISCUSSION**

We have used an electrophoretic mobility shift assay to detect proteins in Xenopus ovary extracts that interact with U8 snoRNA. We have identified a 29-kDa protein that copurifies with U8-binding activity as measured by both gel mobility shift assay and by UV cross-linking to U8 RNA. The specificity of binding has been demonstrated by competition assays using tRNA, 5 S RNA, and U3 and U14 snoRNAs as competitors. Only the U8 snoRNA binds X29 with high specificity. This is the first U8-specific protein identified; it may aid our understanding of how the U8 snoRNA directs the processing of the large subunit RNAs.

To date, U8 is the only vertebrate snoRNA shown to be essential for maturation of both 5.8 and 28 S rRNAs. In contrast, genetic or biochemical depletion of U3, U14, U22, or snR30 results in the absence of mature 18 S rRNA without affecting processing of either 5.8 or 28 S rRNAs (reviewed in Ref. 1). Characterization of U8 snoRNP, particularly its unique components, may allow identification of other factors involved in vertebrate large subunit rRNA processing and factors specific for this portion of the rRNA processing pathway.

**Common (Shared) snoRNA Proteins—**By analogy to other snoRNPs, the U8 particle might be expected to contain both common and unique protein constituents. For example, those snoRNAs containing the C and D box sequence elements associate with three proteins constituting common components of their RNP. One of these, fibrillarin (known as Nop1p in yeast), is very well conserved across evolution (20–24). However, fibrillarin does not appear to directly contact the snoRNA (25), and its association with several snoRNAs is salt-dependent (19, 26). The majority of the snoRNAs that associate with fibrillarin/Nop1p are involved in the site-specific methylation of rRNA (reviewed in Ref. 2). The exceptions are U3, U8, U14, U22, and snR30, which are essential for pre-rRNA processing (reviewed in Ref. 1). The second protein, Nop5/5p, was recently identified as a common C and D box protein required for snoRNA stability in yeast (6). The third shared protein, Nop56, is another yeast protein that binds all the C and D box snoRNAs examined (7). The vertebrate homologues of these proteins have been identified in mouse (11). These shared subunits may have functions common to a variety of particles, so their analysis might not provide insight into function unique to a particular snoRNP. For this reason, we have chosen to focus on identifying proteins unique to U8 snoRNP.

50-fold molar excess of unlabeled sense strand U8 RNA (lane 3) to the binding reaction greatly inhibited complex formation. The inhibition is complete in the presence of a 100-fold molar excess of unlabeled U8 RNA competitor (lane 4). Addition of a 50- or 100-fold molar excess of nonspecific 5 S competitor (lanes 9 and 10) does not affect complex L formation. Lanes 5 and 6 show U8 RNA complex formation in the presence of 50- and 100-fold molar excess, respectively, of cold U3 RNA. The presence of U3 RNA in the binding reaction had no detectable effect on the formation of complex L.
human (DKC1 gene) (32). Mutations in the DKC1 gene in human have been linked to the disease dyskeratosis congenita (29, 32). This example illustrates how characterization of a distinct component of an RNP family can provide insights into the functions of the members of that family and to its distribution in a wide variety of organisms.

The X29 protein reported here has not previously been characterized in Xenopus (based on mass spectrometry analysis and protein size) or in other organisms (based on preliminary protein sequence data). The RNA binding properties of the protein suggest that it may be unique to U8. Thus, learning the identification of U8 homologues in other species may provide information and insight into the functions of the members of that family and to its distribution in a wide variety of organisms.

The mobility gel shift assay performed with crude Xenopus extract clearly indicated several specific complexes could assemble on U8 RNA (see Fig. 1). The two complexes, L and U, were not visible in the crude extract (shown in Fig. 1). However, in the elution profile from the DEAE column (see Fig. 4) they were clearly identified and U8-specific. Thus, gel mobility shift assay has provided evidence for a number of U8-binding proteins. Because U8 is a C and D box snoRNP, we would expect the three common C and D box proteins previously described (reviewed in Ref. 3) to also be part of the U8 RNP. By analogy to other snoRNPs, we would predict the presence of several U8-binding proteins, some shared common proteins, others unique to U8. The U1 small nuclear ribonucleoprotein particle has nine shared common proteins and three proteins unique to U1 (reviewed in Ref. 33). The U3 snoRNP was reported to include seven proteins (34). From the size of U8 in sedimentation gradients (10 S particle in humans) (26), we would predict that approximately six to eight proteins may be present.

The X29 protein described here is the first report of a protein component of a snoRNA involved in processing of the RNAs comprising the large ribosomal subunit. Learning more about the proteins that associate with U8 and identifying the sequence specificity and U8 binding sites of these proteins will provide more insight into how the U8 RNP is assembled and functions. This information could allow us to learn more about other roles and cellular functions of the U8 RNP.

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