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# Molecular Cloning of *Xenopus* Fibrillarin, a Conserved U3 Small Nuclear Ribonucleoprotein Recognized by Antisera from Humans with Autoimmune Disease

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**Autoantibodies against U3 small nuclear ribonucleoprotein are associated with scleroderma autoimmune disease. They were shown to react with fibrillarin, a 34- to 36-kilodalton protein that has been detected in all eukaryotes tested from humans to yeasts. We isolated a 1.6-kilobase cDNA clone encoding fibrillarin from a *Xenopus laevis* cDNA library. The protein contains a 79-residue-long Gly-Arg-rich domain in its N-terminal region and a putative RNA-binding domain with ribonucleoprotein consensus sequence in its central portion. This is the first report of cloning of fibrillarin, and the deduced protein sequence is in agreement with the involvement of the protein in a ribonucleoprotein particle.**

By analogy with other small nuclear RNAs, the U3 small nuclear RNA, which is localized in the nucleolus (24, 29), has been proposed to participate in the maturation of pre-rRNA (2, 8, 24, 28), even if the precise mechanism is still much debated (12, 15, 21). The secondary structure of U3 RNA and the protein composition of the U3 small nuclear ribonucleoprotein (snRNP) particle have been determined (22). One of the protein constituents, a 34-kilodalton (kDa) protein extremely rich in glycine and dimethylarginine (DMA) and with a pI of 8.5, was first described in *Physarum polycephalum* and shown to belong to a nuclear ribonucleoprotein (RNP) complex (5). Later, because of a fortuitous recognition of the protein by antiserum from a patient with scleroderma, the protein was identified in rats, biochemically characterized, and then detected in all eucaryotes (1, 11, 18, 20). It is localized during the interphase exclusively in the fibrillar region of the nucleolus and thus has been named fibrillarin (20).

We previously studied another nucleolar protein, nucleolin, whose localization during the cell cycle resembles that of fibrillarin (10, 19) and which also contains a glycine- and DMA-rich domain (16). In a preliminary Northern (RNA) analysis with total RNA purified from *X. laevis* oocytes, we found that a cDNA probe encoding the Gly-DMA domain of Chinese hamster nucleolin (17) hybridized to a major band of 2.7 kilobases (kb) which corresponds to the *X. laevis* nucleolin mRNA (4). At a lower stringency, two other strongly hybridizing RNAs of 1.8 and 1.6 kb were also detected (Fig. 1, lane A). The same probe was then used to screen an *X. laevis* cDNA library constructed in λ gt10 with poly(A)$^+$ RNA from *X. laevis* oocytes by D. Melton. Among the isolated clones, in addition to those encoding *X. laevis* nucleolin (4), one of them, called λ Xomfib and containing a 1.6-kb insert, had a different structure and was further investigated. When used to probe the same Northern blot, the λ Xomfib probe showed strong hybridization to the 1.8-kb RNA and weaker hybridization to a 2.7-kb RNA (Fig. 1, lane B).

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The Xomfib insert was completely sequenced on both strands by the dideoxy chain termination method (Fig. 2A). The first ATG that could be the initiation codon for the longest open reading frame is CCCATGA. This very rare combination has been found in only two mRNAs encoding lymphokines in a total of 699 vertebrate mRNAs analyzed (14). The open reading frame which follows could encode a 323-amino-acid-long polypeptide with an $M_r$ of 34,330. The TGA stop codon is followed by a 528-nucleotide-long untranslated sequence, and a polyadenylation signal (ATTAAA) is located 19 nucleotides upstream from a poly(A) tract.

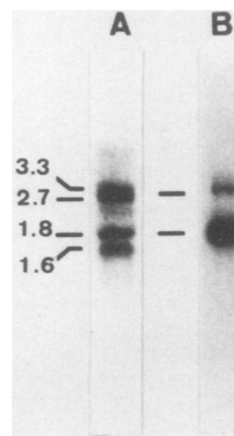


FIG. 1. Northern blot analysis of *Xenopus* RNA with different probes. Total RNA, extracted by standard procedures (23) from two oocytes, was separated by electrophoresis on a denaturing formaldehyde–1% agarose gel, transferred to nitrocellulose, and hybridized with different nick-translated cDNA probes. Hybridization was carried out in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–50% formamide at 42°C for 15 h. Filters were washed twice with 2× SSC–0.1% sodium dodecyl sulfate at room temperature and twice with 0.1× SSC–0.1% sodium dodecyl sulfate at 48°C. Lane A, CHO nucleolin cDNA; lane B, *Xenopus* fibrillarin cDNA. Numbers at left are in kilobases.

A

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                                                 30                                          60                                          90
GAG TGT GTG AGA TCG AGC AGG AGA ATC GCA GCG CGG CCC ATG AGA CCA GGA TTC AGC CCC AGA GGA GGC CGA GGA GGT TTT GGA GAC AGA
                                                    MET Arg Pro Gly Phe Ser Pro Arg Gly Gly Arg Gly Gly Phe Gly Asp Arg    17
                                                 120                                         150                                         180
GGA GGT TTT GGG GGC CGA GGT GGA TTT GGA GAC CGG GGT GGA TTT CGA GGC GGC TCT AGA GGA GGA TTT GGT GGG CGT GGC CGT GGA GGT
Gly Gly Phe Gly Gly Arg Gly Gly Phe Gly Asp Arg Gly Gly Phe Arg Gly Gly Ser Arg Gly Gly Phe Gly Gly Arg Gly Arg Gly Gly    47
                                                 210                                         240                                         270
GAT CGC GGG GGT CGA GGA GGA TTC AGA GGT GGA TTC AGT TCT CCA GGG AGA GGC GGC CCC CGA GGA GGA GGA AGA GGA GGA TTT GGA GGA
Asp Arg Gly Gly Arg Gly Gly Phe Arg Gly Gly Phe Ser Ser Pro Gly Arg Gly Gly Pro Arg Gly Gly Gly Arg Gly Gly Phe Gly Gly    77
                                                 300                                         330                                         360
GGA AGA GGA GGA TTT GGA GCA GGA AGG AAA GTG ATA GTA GAG CCT CAC AGA CAT GAA GGA ATC TTC ATC TGC CGA GGG AAG GAA GAC GCT
Gly Arg Gly Gly Phe Gly Ala Gly Arg Lys Val Ile Val Glu Pro His Arg His Glu Gly Ile Phe Ile Cys Arg Gly Lys Glu Asp Ala   107
                                                 390                                         420              a                          450
CTG GTG ACC AAA AAC CTT GTT CCT GGG GAG TCT GTG TAT GGG GAG AAA AGG ATC TCA GTG GAG GAC GGG GAA GTG AAG ACG GAA TAC AGA
Leu Val Thr Lys Asn Leu Val Pro Gly Glu Ser Val Tyr Gly Glu Lys Arg Ile Ser Val Glu Asp Gly Glu Val Lys Thr Glu Tyr Arg   137
                                                 480                                         510                                         540
GCC TGG AAT CCT TTC AGG TCC AAG ATT GCG GCG GCC ATT CTG GGA GGA GTC GAT CAG ATT CAC ATT AAG CCG GGA GTT AAA GTT CTG TAT
Ala Trp Asn Pro Phe Arg Ser Lys Ile Ala Ala Ala Ile Leu Gly Gly Val Asp Gln Ile His Ile Lys Pro Gly Val Lys Val Leu Tyr   167
                                                 570                                         600                                         630
CTG GGG GCG GCG TCA GGA ACC ACC GTC TCT CAC GTC TCT GAT GTC GTG GGA CCT GAG GGG CTG GTG TAC GCC GTC GAG TTC TCC CAC AGA
Leu Gly Ala Ala Ser Gly Thr Thr Val Ser His Val Ser Asp Val Val Gly Pro Glu Gly Leu Val Tyr Ala Val Glu Phe Ser His Arg   197
                                                 660                                         690                                         720
TCC GGC CGT GAT CTC ATA AAC GTG GCG AAG AAA CGG ACG AAT ATC ATT CCG GTG ATT GAA GAC GCC CGG CAC CCC CAC AAG TAC CGC ATA
Ser Gly Arg Asp Leu Ile Asn Val Ala Lys Lys Arg Thr Asn Ile Ile Pro Val Ile Glu Asp Ala Arg His Pro His Lys Tyr Arg Ile   227
                                                 750                                         780                                         810
TTA GTG GGA ATG GTG GAC GTG GTC TTT GCA GAC GTG GCT CAA CCT GAT CAG ACC AGG ATC GTC GCC CTC AAC GCC CAT AAC TTC CTA AAG
Leu Val Gly MET Val Asp Val Val Phe Ala Asp Val Ala Gln Pro Asp Gln Thr Arg Ile Val Ala Leu Asn Ala His Asn Phe Leu Lys   257
                                                 840                                         870                                         900
AAC GGA GGC CAC TTT GTC ATA TCC ATC AAG GCA AAC TGC ATT GAC TCC ACG GCA GCC CCA GAG GCA GTT TTT GCT GCG GAA GTG AAG AAG
Asn Gly Gly His Phe Val Ile Ser Ile Lys Ala Asn Cys Ile Asp Ser Thr Ala Ala Pro Glu Ala Val Phe Ala Ala Glu Val Lys Lys   287
                                                 930                                         960                                         990
ATG CAA CAA GAG AAC ATG AAG CCG CAG GAG CAG CTG ACG CTG GAG CCG TAC GAG AGA GAT CAC GCC GTG GTG GTC GGG ATT TAC AGG CCG
MET Gln Gln Glu Asn MET Lys Pro Gln Glu Gln Leu Thr Leu Glu Pro Tyr Glu Arg Asp His Ala Val Val Val Gly Ile Tyr Arg Pro   317
                                                          1050                                          1100
CCG CCC AAA CAG AAG AAA TGA TGAGAAGGAGCAGGATGAAGATTTGTTTTAAAGGACTTTTATTATACGAGAACTTTCCCAGCCAGAGACTGACGTGAATGGGTTAATGACG
Pro Pro Lys Gln Lys Lys ---                                                                                               323
                                            1150                                           1200
CCGGGATTTAACCATCGCAATGTACAGCAACTCTGTGGCCTTTTAGAATGGAGGAGGGGGCGGGACTTGTGGCCCCTCAGCTGTCGTGAGCTGAAACTGCTGGGAGTTGCCGGGAGTTG
                               1250                                           1300
CAGCTCATCTCAGCTGGGGGGGGGCCTTCAAGTTGTTGTTTCTGCTCTACCTGAGGCCACAGACCATTATTTTATAGATTTTTTTTTTTTTTTATATTCAACGATTAACCACCATATTA
        1350                                            1400                                          1450
AGATGCCCCACCCCTCTTTCATTGGCCACTCCCCCTGCGTCGTATAATGGCGTCTCCATAACAACGTACCAGTTTTCCCTGCACCCGGGGGGAGGGGCAGATTCTATGGGAAGTGCAAG
                                        1500                                           1550
AGAAGTGATTTTTTTTATGTTTTTTAAGAAAGAAAAACTTGAAAATGAACCAGAAAAAAGAGATGGAAATTCTTATTTTAATTAAAGCGACAGTGTGTGAAACCGAAAAAAAAAAAAAA
                    1600
AAAAAAAAAAAAAAAAAAAAAA
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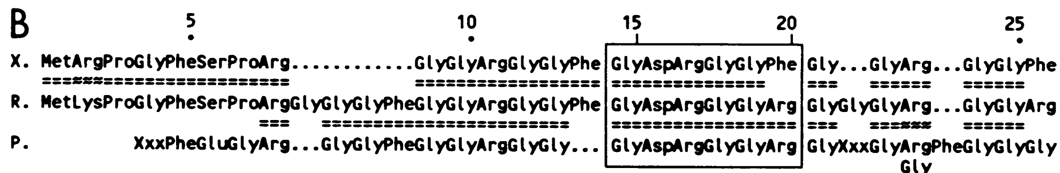


FIG. 2. (A) Nucleotide sequence of the Xomfib clone encoding fibrillarin. The translated sequence of the longest open reading frame is indicated. The entire Gly-DMA-rich domain is thinly underlined, with arginines denoted by asterisks. A 12-residue motif is tandemly repeated two times (positions 9 to 20 and 21 to 32). The six-residue-long motif which contains an aspartic acid is boxed (thin lines). It is repeated three times in *Xenopus* fibrillarin and is also found once in each available sequence for rat and *Physarum* fibrillarins. The putative RNA-binding domain of *Xenopus* fibrillarin is heavily underlined, and the RNP consensus sequence is boxed (heavy lines). The poly (A) addition signal is indicated (double dashed lines) 19 nucleotides upstream of the poly (A) tract. The TGA stop codon is indicated by a broken line. (B) Alignment of the sequence of *Xenopus* fibrillarin (X) with the available N-terminal sequences of rat (R) (18) and *Physarum* (P) (6) fibrillarins obtained by direct sequencing of these proteins. Gaps were allowed to produce the best matches among the three species. Perfect matches are denoted by broken lines, conservative substitutions are denoted by wavy broken lines, and three positions in *Physarum* fibrillarin that are uncertain are denoted as Xxx and Arg/Gly. The boxed motif is highy conserved and is present three times in *Xenopus* fibrillarin.

Two lines of evidence demonstrated that the Xomfib cDNA encoded fibrillarin. First, a comparison with the N-terminal sequences of rat (18) and *Physarum* (6) fibrillarins, both obtained by direct peptide sequencing (31 and 25 residues, respectively), revealed strong sequence homologies, except for deletions or insertions of short blocks of residues which can frequently occur in Gly-DMA domains, as already shown for nucleolin (4, 17). When the sequences were aligned, the homology in the N-terminal part of fibrillarin from the two vertebrates was around 90% (Fig. 2B). It would appear that this type of domain evolves quickly from one species to another by duplication of some basic elements, leading to an increase in the size of the domain. This conclusion is supported by the observation that the N terminus of the Gly-rich domain of *Xenopus* fibrillarin exhibits a perfect tandem repeat of 12 residues (Fig. 2A). The Gly-rich domains of the different proteins share neither exactly the same structure nor the same residue composition. For example, the Gly-rich domain of CHO nucleolin contains only Gly, Arg, or Phe residues, while in *Xenopus* fibrillarin, the domain also contains three Asp, three Ser, two Pro, and one Ala residue(s). Strikingly, the three Asp residues are each part of another repeat that is present three times in *Xenopus* fibrillarin and that is also found in the available sequences for rat and *Physarum* fibrillarins. This finding suggests that these residues may have an important function.

A second approach to identify the recombinant clone was to take advantage of the fact that antiserum S4, from a patient with an autoimmune disease (a gift from R. Lürhmann), recognizes fibrillarin from very different species. We first checked the specificity of this antiserum in a Western blot (immunoblot) experiment with nuclear proteins from CHO cells (Fig. 3A, lane 1) or *Xenopus* hepatocytes (lane 2). In both species, fibrillarin was specifically recognized by the human antifibrillarin antibodies. We investigated whether the recombinant protein produced in an *Escherichia coli* host strain harboring Xomfib cDNA cloned downstream of a T7 polymerase promoter (27) could also be recognized. Unexpectedly, the recombinant protein was not recognized by the antiserum (data not shown). We then prepared in vitro a synthetic capped mRNA by transcription of the cloned cDNA with T7 RNA polymerase. Translation of this RNA in a rabbit reticulocyte lysate yielded a major labeled protein of 34 kDa (Fig. 3B, lane 1), the size of the *Xenopus* fibrillarin detected by the antiserum. Figure 3B (lanes 2 and 3) and Fig. 3C show the labeled proteins immunoprecipitated by antisera S70 and S4 from the in vitro translation system. To remove any nonspecific reaction and also as a negative control, we carried out preliminary immunoprecipitation with serum that was from a patient with another autoimmune disease and that contained antibodies directed against a 70-kDa nuclear protein (S70). No labeled protein was immunoprecipitated with this antiserum (Fig. 3B, lane 2), whereas when the protein A-Sepharose-coupled S4 antiserum was incubated with the supernatant of the preliminary immunoprecipitation and extensively washed, as described by R. Reuter (26), the 34-kDa protein was found associated with S4 antibodies (Fig. 3B, lane 3). A longer exposure is shown in Fig. 3C. Thus, the Xomfib clone can direct the synthesis of a 34-kDa peptide either in *E. coli* or in vitro in a rabbit reticulocyte lysate, but only the latter peptide could be detected by the human antiserum that recognizes fibrillarin. The possibility that this protein might undergo posttranslational modifications not performed by the *E. coli* machinery

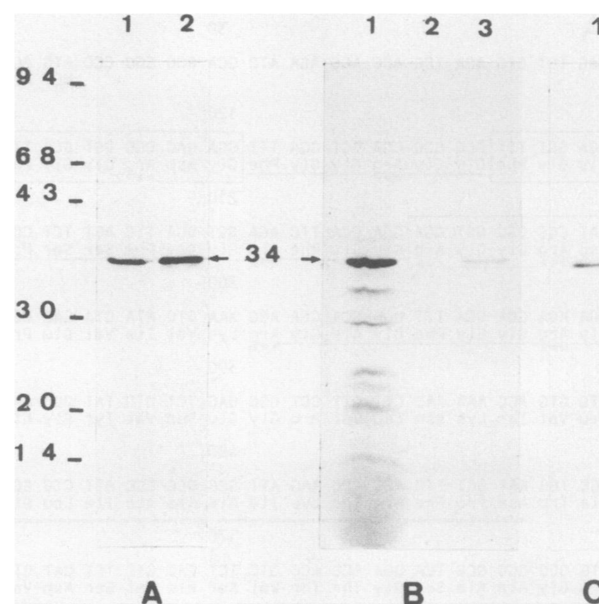


FIG. 3. Characterization of fibrillarin and identification of the recombinant protein. (A) Nuclei from either CHO cells (lane 1) or *Xenopus* hepatocytes (lane 2) were prepared, and total protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Western blotted to nitrocellulose, and probed with the S4 antiserum. (B and C) A synthetic RNA was prepared from the recombinant fibrillarin cDNA clone by using the T7 transcription system and translated in a rabbit reticulocyte lysate containing [$^{35}$S] methionine to monitor protein synthesis (lane 1). Two successive immunoprecipitations were then performed as described by R. Reuter (26) with the lysate product and two different antisera, and each precipitate was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. First, total lysate was incubated with protein A-Sepharose coupled to autoimmune antiserum from a patient who had an autoimmune disease and whose serum did not react with fibrillarin (lane 2). The supernatant from this step was then incubated with S4 auto-antiserum–protein A-Sepharose (lane 3). Panel C shows an exposure three times longer than that shown in panel B, lane 3. Values to the left of the gel are in kilodaltons.

but possibly involved in the recognition of epitopes by patient antisera cannot be excluded.

Taken together, these results infer that we have isolated and characterized a cDNA encoding fibrillarin, a widespread auto-antigen from patients with scleroderma disease. Although it is possible that additional forms of fibrillarin are present in *Xenopus* somatic cells, there is currently no evidence for this.

An RNP consensus motif of eight residues, RNP1, has been identified in a 90-amino-acid domain from different RNA-binding proteins (9). This domain was recently shown to bind directly to specific RNA sequences and thus represents the RNA recognition motif characteristic of a distinct family of proteins (25). In fibrillarin, residues 184 to 195 exhibit some homology to this consensus motif, and the score is higher in comparison with other snRNPs rather than with heterogeneous nuclear RNPs (hnRNPs) or preribosomal RNP (pre-rRNP) particles. For instance, as in the case of two of three snRNPs, fibrillarin does not possess an aromatic residue at the third position of the RNP1 consensus motif. We have considered the whole domain of about 90 residues, and an alignment with the three RNA-binding domains of snRNPs is proposed in Fig. 4. Of 88 residues, 28

```
U1A     AVPETRPNHTIYINNLNEKIKKD.ELKKSLYAIF.SQF.GQILDILVSR.SLK..M |RGQAF.VIF| KEVSS...ATN.ALRSMQGFP.FYD.KPMRIQYAKT
          ≈ ===============■≈=====≈ ===+=====■= === =±■■===■  +  ■=  = |===== ===| ===■ =    == === ■==== ==  ==========
U1B       MDIRPNHTIYINNMNDKIKKE.ELKRSLYALF.SQF.GHVVDIVALK.TMK..M |RGQAF.VIF| KELGS...STN.ALRQLQGFP.FYG.KPMRIQYAKT
          ±     ==■≈■  :=        ==≈  ≈ ■≈      ±■  ■   ≈   =  |== == ■ ≈|              = ≈± ±=     = ≈ ■ ■±  ≈
U1-70K  PNAQGDAFKTLFVARVNYDTTES.KLRRE.FEVY.GPI.KRIHMVYSKR.SGK..P |RGYAF.IEY| EHE..RD.MHS.AYKHADGKK.IDG.RRVLVDVERG

Fibril        NPFRSKIAAAILGGV.DQIHIKPGVKVLYLGAASGTT....VSHVSDVVGP |EGLVYAVEF| SHRSGRDLI.NVAKKRTNIIPVIEDARHPH.KY.RI
              ± ==≈  ■= ■   ■ ±± ±■=  ■ ■== =■■ =      ==≈ =     = |±=■■≈ ===| = = == ■ = = =± ±■■= ==≈ =±■± ±= =■
```

FIG. 4. Alignment of the putative RNA-binding domain of fibrillarin with those of other snRNPs: U1A, U1B, and U1-70K (for a review, see reference 3). Fibril, Fibrillarin residues 140 to 230. The RNA-binding consensus motif is boxed. Some gaps have been allowed to provide the best matches. For fibrillarin a compilation was established as follows: when a position in fibrillarin was found to be identical (broken lines), a conservative substitution (wavy lines), or occupied by a charged amino acid (±) or by a hydrophobic residue (■) in any of the other proteins, the result is indicated under the fibrillarin sequence.

are strictly conserved in at least one snRNP, i.e., 32%, while the score reaches 66% if we also consider the conservative substitutions. This domain may be responsible for the binding between fibrillarin and U3 RNA. The relatively poor homology to the sequences of proteins from other classes of RNP (messenger RNP, hnRNP, and pre-rRNP) raises the possibility that the divergence may result from a coordinate evolution of both the protein and its RNA target. Another structural feature of some RNA-binding proteins is the presence of an auxiliary domain (3). Until now, in the RNP family, if we set aside SSB1 protein (13), whose involvement in RNPs is not established, only nucleolin, the hnRNP protein A1 and fibrillarin possess a Gly-DMA domain. This domain, which was shown to have a cooperative effect in RNP formation (7), could interact directly with RNA or be involved in protein-protein interactions with RNPs or matrix proteins.

Besides the study of its functional role(s) in ribosome biogenesis and its relationship to the other components of snRNP particles during *Xenopus* development, the establishment of the primary structure of fibrillarin will allow investigation of its involvement in autoimmune disorders.

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## LITERATURE CITED


1. **Aris, J. P., and G. Blobel.** 1988. Identification and characterization of a yeast nucleolar protein that is similar to a rat liver nucleolar protein. J. Cell Biol. **107:**17–31.
2. **Bachellerie, J. P., B. Michot, and F. Raynal.** 1983. Recognition signals for mouse pre-rRNA processing. Mol. Biol. Rep. **9:**79–86.
3. **Bandziulis, R. J., M. S. Swanson, and G. Dreyfuss.** 1989. RNA-binding proteins as developmental regulators. Genes Dev. **3:**431–437.
4. **Caizergues-Ferrer, M., P. Mariottini, C. Curie, B. Lapeyre, N. Gas, F. Amalric, and F. Amaldi.** 1989. Nucleolin from Xenopus laevis: cDNA cloning and expression during development. Genes Dev. **3:**324–333.
5. **Christensen, M. E., A. L. Beyer, B. Walker and W. M. LeStourgeon.** 1977. Identification of $N^G$, $N^G$-dimethylarginine in a nuclear protein from the lower eukaryote *Physarum polycephalum* homologous to the major proteins of mammalian 40S ribonucleoprotein particles. Biochem. Biophys. Res. Commun. **74:**621–629.
6. **Christensen, M. E., and K. P. Fuxa.** 1988. The nucleolar protein, B-36, contains a glycine and dimethylarginine-rich sequence conserved in several other nuclear RNA-binding proteins. Biochem. Biophys. Res. Commun. **155:**1278–1283.
7. **Cobianchi, F., R. L. Karpel, K. R. Williams, V. Notario, and S. H. Wilson.** 1988. Mammalian heterogeneous nuclear ribonucleoprotein complex protein A1. J. Biol. Chem. **263:**1063–1071.
8. **Crouch, R. J., S. Kanaya, and P. L. Earl.** 1983. A model for the involvement of the small nucleolar RNA (U3) in processing eukaryotic ribosomal RNA. Mol. Biol. Rep. **9:**75–78.
9. **Dreyfuss, G., M. S. Swanson, and S. Piñol-Roma.** 1988. Heterogeneous nuclear ribonucleoprotein particles and the pathway of mRNA formation. Trends Biochem. Sci. **13:**86–91.
10. **Gas, N., M. L. Escande, and B. Stevens.** 1985. Immunolocalization of 100 kDa nucleolar protein during mitotic cycle in CHO cells. Biol. Cell. **53:**209–218.
11. **Guiltinan, M. J., M. E. Schelling, N. Z. Ehtesham, J. C. Thomas, and M. E. Christensen.** 1988. The nucleolar RNA-binding protein B-36 is highly conserved among plants. Eur. J. Cell Biol. **46:**547–553.
12. **Jeppesen, C., B. Stebbins-Boaz, and S. A. Gerbi.** 1988. Nucleotide sequence determination and secondary structure of Xenopus U3 snRNA. Nucleic Acids Res. **16:**2127–2148.
13. **Jong, A. Y. S., M. W. Clark, M. Gilbert, A. Oehm, and J. L. Campbell.** 1987. *Saccharomyces cerevisiae* SSB1 protein and its relationship to nucleolar RNA-binding proteins. Mol. Cell. Biol. **7:**2947–2955.
14. **Kozak, M.** 1987. An analysis of 5′ noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res. **20:**8125–8148.
15. **Kupriyanova, N. S., and M. Y. Timofeeva.** 1988. 32S pre-rRNA processing: a dynamic model for interaction with U3 RNA and structural rearrangements of spacer regions. Mol. Biol. Rep. **13:**91–96.
16. **Lapeyre, B., F. Amalric, S. H. Ghaffari, S. V. Venkatarama Rao, T. S. Dumbar, and M. O. J. Olson.** 1986. Protein and cDNA sequence of a glycine-rich, dimethylarginine containing region located near the carboxyl-terminal end of nucleolin (C23 and 100 kDa). J. Biol. Chem. **261:**9167–9173.
17. **Lapeyre, B., H. M. Bourbon, and F. Amalric.** 1987. Nucleolin, the major nucleolar protein of growing eukaryotic cells: an unusual protein structure revealed by the nucleotide sequence. Proc. Natl. Acad. Sci. USA **84:**1472–1476.
18. **Lischwe, M. A., R. L. Ochs, R. Reddy, R. G. Cook, L. C. Yeoman, E. M. Tan, M. Reichlin and H. Busch.** 1985. Purification and partial characterization of a nucleolar schleroderma antigen (Mr = 34,000; pI, 8.5) rich in $N^G$, $N^G$-dimethylarginine. J. Biol. Chem. **260:**14304–14310.
19. **Ochs, R. L., M. A. Lischwe, E. Shen, R. E. Carroll, and H. Busch.** 1985. Nucleologenesis: composition and fate of prenucleolar bodies. Chromosoma **92:**330–336.
20. **Ochs, R. L., M. A. Lischwe, W. H. Spohn, and H. Busch.** 1985. Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. Biol. Cell. **54:**123–134.
21. **Parker, K. A., J. P. Bruzik, and J. A. Steitz.** 1988. An *in vitro* interaction between the human U3 snRNP and 28S rRNA sequences near the α-sarcin site. Nucleic Acids Res. **16:**10493–10509.
22. **Parker, K. A., and J. A. Steitz.** 1987. Structural analyses of the human U3 ribonucleoprotein particle reveal a conserved sequence available for base pairing with pre-rRNA. Mol. Cell.

Biol. **7**:2899–2913.
23. **Pierandrei-Amaldi, P., N. Campioni, E. Beccari, I. Bozzoni, and F. Amaldi.** 1982. Expression of ribosomal protein genes in Xenopus laevis development. Cell **30**:163–171.
24. **Prestayko, A. W., M. Tonato, and H. Busch.** 1970. Low molecular weight RNA associated with 28S nucleolar RNA. J. Mol. Biol. **47**:505–515.
25. **Query, C. C., R. C. Bentley, and J. D. Keene.** 1989. A common RNA recognition motif identified within a defined U1 RNA binding domain of the 70K U1 snRNP protein. Cell **57**:89–101.
26. **Reuter, R., G. Tessars, H. W. Vohr, E. Gleichmann, and R. Lührmann.** 1989. Mercuric chloride induces autoantibodies against U3 small nuclear ribonucleoprotein in susceptible mice. Proc. Natl. Acad. Sci. USA **86**:237–241.
27. **Studier, F. W., and B. A. Moffatt.** 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. **189**:113–130.
28. **Tague, B. W., and S. A. Gerbi.** 1984. Processing of the large rRNA precursor: two proposed categories of RNA-RNA interactions in eukaryotes. J. Mol. Evol. **20**:362–367.
29. **Weinberg, R. A., and S. Penman.** 1968. Small molecular weight monodisperse nuclear RNA. J. Mol. Biol. **38**:289–304.