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Structural Requirements of 5S rRNA for Nuclear Transport, 7S Ribonucleoprotein Particle Assembly, and 60S Ribosomal Subunit Assembly in *Xenopus* Oocytes

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Structural requirements of 5S rRNA for nuclear transport and RNA-protein interactions have been studied by analyzing the behavior of oocyte-type 5S rRNA and of 31 different in vitro-generated mutant transcripts after microinjection into the cytoplasm of *Xenopus* oocytes. Experiments reveal that the sequence and secondary and/or tertiary structure requirements of 5S rRNA for nuclear transport, storage in the cytoplasm as 7S ribonucleoprotein particles, and assembly into 60S ribosomal subunits are complex and nonidentical. Elements of loops A, C, and E, helices II and V, and bulged and hinge nucleotides in the central domain of 5S rRNA carry the essential information for these functional activities. Assembly of microinjected 5S rRNA into 60S ribosomal subunits was shown to occur in the nucleus; thus, the first requirement for subunit assembly is nuclear targeting. The inhibitory effects of ATP depletion, wheat germ agglutinin, and chilling on the nuclear import of 5S rRNA indicate that it crosses the nuclear envelope through the nuclear pore complex by a pathway similar to that used by karyophilic proteins.

The orchestration of ribosome biogenesis in eukaryotic cells is a process that requires transfer of macromolecules into and out of the nucleus. In *Xenopus* oocytes, 5S rRNA is shuttled between the nuclear and cytoplasmic compartments of the oocyte during different stages of oogenesis in a complex pathway involving different protein associations. In previtellogenic oocytes, 5S rRNA is synthesized before other components of ribosomes are available, is exported from the nucleus, and stored in the cytoplasm as 7S ribonucleoprotein particles (RNPs) (5S rRNA complexed with transcription factor IIIA [TFIIIA]) or as 42S RNPs (5S rRNA complexed with other nonribosomal proteins and tRNA). During vitellogenesis, the 5S rRNA is released from storage and a 5S rRNA-ribosomal protein L5 complex, which is a precursor to assembly into the 60S large ribosomal subunit, forms (reference 3 and references therein). We are interested in the mechanisms that govern the subcellular trafficking of 5S rRNA within the oocyte, particularly the requirements for the mobilization of stored 5S rRNA during ribosome assembly.

There is ample evidence that transit of RNA and RNPs into and out of the nucleus occurs exclusively via the nuclear pores (20, 45; for a review, see reference 30). Although nuclear localization of proteins has been well characterized (1, 10, 21, 49; for a review, see reference 61), the factors governing nuclear export and import of RNA and RNPs remain enigmatic. Nuclear export of tRNA (75), pre-small nuclear RNAs (50), mRNA (16), 40S and 60S ribosomal subunits (6, 38), and 5S rRNA (24) occurs in a manner consistent with a mediated process. Analysis of the nuclear transport of U small nuclear RNAs has multiple defined, kinetically distinct targeting pathways (27, 47, 48). Nuclear transport of different classes of RNA may thus involve targeting to the pore complex by different cytoplasmic receptors and then translocation into the nucleus by the same pore complex-mediated mechanism. Specific RNA structures have been implicated as requirements for both nuclear import and export (5, 22, 32, 33, 67, 75). Translocation of RNA molecules across the nuclear envelope may also require interaction with specific proteins (28, 31, 33, 43, 62).

RNA-protein interactions are important for many regulatory processes. There is growing evidence that RNA structures, such as helices, loops, bulges, mismatches, and pseudoknots, are key elements in protein recognition (for a review, see reference 19). The sequence and structural requirements for binding of TFIIIA to 5S rRNA have been the subjects of extensive research (7, 8, 56, 66, 74). In comparison, the structural elements of the 5S rRNA molecule required for assembly into ribosomes and for its functional activity within the ribosome remain to be elucidated (34, 37, 69).

We report here the results of a structural analysis of 31 different 5S rRNA mutants by nuclear transport and RNP assembly assays in *Xenopus* oocytes. The results are discussed in relation to the abilities of these same 5S rRNA mutants to bind TFIIIA in vitro (7, 8, 56, 74). We show that determinants of nuclear transport, TFIIIA binding, and ribosome incorporation within the 5S rRNA molecule are complex and nonidentical. The results suggest that TFIIIA binding is not a prerequisite for nuclear targeting of 5S rRNA, consistent with earlier studies indicating that 7S RNPs are not imported (3, 44). We show that microinjected

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5S rRNA is incorporated into 60S ribosomal subunits in the nucleus; thus, the first requirement for assembly is nuclear import. Finally, we demonstrate that nuclear import of 5S rRNA is sensitive to general inhibitors of nuclear pore-mediated translocation.

MATERIALS AND METHODS

Synthesis of mutant 5S rRNAs. The 5S rRNA genes used in these experiments were constructed from a series of synthetic oligonucleotides that were subsequently introduced into pUC18 as previously described (56, 58). Internally labelled 5S rRNAs were produced by in vitro transcription from these gene templates with T7 RNA polymerase (Boehringer Mannheim N.Z. Ltd., Auckland, New Zealand) and 50 to 100 μCi of [α-^{32}P]GTP (3,000 Ci/mmol; Amersham Australia Pty Ltd., Auckland, New Zealand). The mixture was incubated for 1.5 h at 37°C and then treated with RNase-free DNase (Boehringer Mannheim). The sample was extracted with phenol and chloroform, and the RNA transcripts were precipitated twice with 2.5 M ammonium acetate and ethanol. The RNA pellet was resuspended in TE (10 mM Tris-HCl, 1 mM EDTA), pH 7.6, and stored at −80°C. RNA concentration was quantified by DNA Dipstick (Invitrogen Corp., San Diego, Calif.), according to the manufacturer's instructions.

Microinjection and analysis of nuclear transport. An ovary lobe was surgically removed from an adult *Xenopus laevis* (reared at the University of Canterbury on a diet of cockroaches), and the oocytes were separated by brief collagenase treatment as previously described (3). Stage 5-6 oocytes were microinjected with 40 to 80 nl of RNA (0.2 to 0.5 ng of RNA per oocyte) into the oocyte cytoplasm with a PV 830 PicoPump (World Precision Instruments, Inc., New Haven, Conn.) by previously published procedures (3). After overnight incubation (18 h) in O-R2 medium (3), nuclei were manually dissected from oocytes in 1% trichloroacetic acid and collected for analysis (3). RNA was extracted from nuclear and cytoplasmic fractions as described by Allison et al. (3). RNA was analyzed by 8% polyacrylamide–8 M urea gel electrophoresis as described previously (3). Dried gels were autoradiographed on Amersham Hyperfilm-MP at −80°C. A Kontron Uvikon 860 spectrophotometer equipped with a gel scan accessory was used to measure the intensity of bands on suitable exposures of autoradiograms (within the linear range of signal intensity of the film); data were quantified with software package 8543. The system has high resolution, and the software accommodates background levels and irregularly shaped bands. Only mutants that showed less than 50% nuclear transport relative to oocyte-type 5S rRNA were considered to be significantly impaired.

For ATP depletion assays, oocytes were preinjected with 50 nl of 1-U/μl apyrase (grade VIII; Sigma Chemical Co., St. Louis, Mo.) in phosphate-buffered saline (PBS) (3) to give a final intracellular concentration of 100 U/ml. Alternatively, 50 nl of PBS was preinjected as a control. After 30-min incubation at 18°C, oocytes were injected with labelled RNA. After an additional 6-h incubation, oocytes were analyzed for nuclear import as described above. ATP depletion was verified with a CLS ATP bioluminescence kit (Boehringer Mannheim). Single oocytes were homogenized in boiling 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.5, and incubated for 5 min at 100°C, and samples were then diluted 1:500 in double-distilled water. A 500-μl sample was added to an equal volume of luciferase extract immediately prior to measurement of luminescence with an SA1 ATP photometer (model 2000).

Wheat germ agglutinin (WGA) (Sigma Chemical Co.) was dissolved in PBS at concentrations ranging from 0.25 to 2 mg/ml. WGA (50 nl) or PBS (as a control) was injected into the oocyte cytoplasm. After incubation for 3 h, oocytes were injected with labelled RNA and analyzed for nuclear import as described above. As a control for the specificity of preinjection of 50 nl of 1-mg/ml WGA, 50 nl of 1-mg/ml WGA and 500 mM *N*-acetylglucosamine (Sigma Chemical Co.) were coinjected into oocytes prior to injection of labelled 5S rRNA. As a control for nonselective diffusion, oocytes were injected with 50 nl of 100-mg/ml fluorescein isothiocyanate-dextran (molecular weight, 10,000 or 150,000; Sigma Chemical Co.). After overnight incubation, nuclei were dissected out in nucleus isolation buffer (3) and viewed by fluorescence microscopy for diffusion of the dextran into the nucleus.

Since there can be variability in synthetic activity between different batches of oocytes, experiments were repeated a minimum of two times with oocytes from different animals.

Antisera. The antibodies raised against TFIIIA have been shown to react with the relevant protein; no cross-reaction with any other protein was noted by immunoblotting (70). Preparation of the antibodies raised against *Xenopus* 60S ribosomal subunits is described by Viel et al. (70); however, there is a slight difference in the reaction of the anti-60S ribosomal subunit antiserum used in this study (anti-serum number 2679; sampling date, 7 November 1985) and of the antiserum described by Viel et al. (70). Immunoblot analysis was performed on total protein from ribosomal subunits purified from mature *Xenopus* ovaries by ultracentrifugation techniques (70) or from 7S fractions obtained after sucrose density centrifugation of cell homogenates of *Xenopus* ovaries (41). The proteins were fractionated by polyacrylamide gel electrophoresis and either stained with Coomassie blue or transferred to Immobilon-P polyvinylidene difluoride membranes (Millipore S.A., Saint-Quentin Yvelines, France). Membranes were incubated with diluted antiserum (1:287) in a solution of 10 mM sodium phosphate (pH 7.4), 150 mM NaCl, and 1% bovine serum albumin, washed in the same buffer, treated with ^{35}S-labelled-protein A in order to label the antigen-antibody complexes, washed again, and exposed to X-ray films at −80°C by previously published procedures (70).

To further ensure that the anti-60S antiserum did not cross-react with 7S RNPs, immunoprecipitation analyses were performed on 7S RNPs purified from immature *Xenopus* ovaries as previously described (3). Immunoprecipitation assays were performed as described by Allison et al. (3), using approximately 10 μg of 7S RNPs and 10 μl of antiserum bound to protein A-Sepharose (Pharmacia LKB Biotechnology, Auckland, New Zealand). Immunoprecipitates were vortexed briefly in sample buffer (2% sodium dodecyl sulfate, 100 mM dithiothreitol, 60 mM Tris [pH 6.8], 0.01% bromophenol blue), heated at 100°C for 5 min, and then centrifuged for 30 s. Proteins from the supernatant were resolved by polyacrylamide gel electrophoresis and silver stained with a Bio-Rad silver stain kit (Bio-Rad Laboratories Pty Ltd., Auckland, New Zealand), according to the manufacturer's instructions.

Immunoprecipitation assays. Microinjected oocytes were incubated for 48 h at 18°C. Immunoprecipitation assays were performed as described by Allison et al. (3), using homogenates of 20 microinjected oocytes and 10 μl of antiserum bound to protein A-Sepharose. For nuclear immunoprecipitations, 20 nuclei were isolated in nucleus isolation buffer

(3). RNA was recovered from the immunoprecipitates and immunosupernatants and resolved on 8% polyacrylamide–8 M urea gels, and then autoradiography was performed. Results were interpreted qualitatively by comparing the amounts of the different mutants immunoprecipitated with the amount of oocyte-type 5S rRNA immunoprecipitated and by comparing the relative specific activities of the RNA mutants and the amounts of RNA injected, using the supernatant fractions.

Electrophoretic mobility shift assays. After a 48-h incubation at 18°C, five microinjected oocytes were homogenized in 20 μl of RNP homogenization buffer (20 mM Tris [pH 7.6], 100 mM KCl, 1.5 mM $MgCl_2$, 2 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 10 U of RNasin per ml; Promega:Pacific Diagnostics Pty. Ltd., Auckland, New Zealand). A crude cellular lysate was prepared by disrupting oocytes with a Gilson tip, spinning down the cellular debris (yolk and pigment) by centrifugation at 9,000 × *g* for 10 min at 4°C, and removing the supernatant, taking care not to disturb the lipid pellicle floating on the surface. Glycerol-dye loading buffer was added to the supernatant, and samples were loaded directly onto a 6% polyacrylamide–0.1% Triton X-100 gel in Tris-borate-EDTA buffer plus 0.1% Triton X-100. Samples were electrophoresed at 300 V; gels were dried at 80°C for 30 min, and then autoradiography was performed. A sample of unlabelled, native 7S RNPs were included as a marker. The marker lane was stained with ethidium bromide and viewed by UV illumination.

RESULTS

Sequence and structural requirements for nuclear transport of 5S rRNA. The sequence and structural requirements of 5S rRNA for nuclear transport in *Xenopus* oocytes were investigated by analyzing 31 mutant 5S rRNA molecules generated by in vitro transcription. Figure 1 shows the secondary structure of oocyte-type 5S rRNA and the locations of the mutations analyzed. With the exception of seven mutants, substitutions were located between nucleotides 11 and 108 in the region of the RNA molecule shown to provide the necessary sequence and conformational information required for nuclear transport (3). In our previous study, however, we did not investigate quantitative differences in nuclear transport.

All mutant 5S rRNAs were stable 24 h after cytoplasmic microinjection (data not shown). Analysis of oocyte-type 5S rRNA revealed that on average, 14% of the microinjected RNA was found in the nucleus after 18 h (Fig. 2, lanes 1 and 2). These results were consistent with a previous study, which demonstrated that the total amount of labelled 5S rRNA found in the nucleus increases for up to 18 to 21 h and then remains constant (3). Presumably, this constant amount reflects steady-state levels of 5S rRNA molecules which have migrated into the nucleus and are being assembled into ribosomes. Nuclear transport of 5S rRNA thus represents a state of flux between the cytoplasm and nucleus, as opposed to a measure of nuclear import alone. The 31 mutant 5S rRNAs showed a variety of transport phenotypes. The intracellular distribution of five mutant RNAs is presented in Fig. 2. The most defective mutant, 16–21, showed a 75% reduction in nuclear transport relative to that of oocyte-type 5S rRNA (lanes 3 and 4). Mutants 41–44 and 105–108 were less defective in nuclear transport, showing reductions of 67 and 54%, respectively (compare lanes 5 and 6 and lanes 11 and 12). In contrast, mutants Δ63 and 67–70 had transport characteristics similar to those for oocyte-type 5S rRNA (lanes 7 to 10). The data are summarized in Table 1.

Mutations within predicted helices II and V tend to have lower nuclear transport values if the mutations are helix breaking (HB in Table 1), than if the mutations are helix maintaining (HM). Nucleotide substitutions that alter either the 5′ sequence of helix II or the sequence and structure of helix V resulted in a significant reduction (≥50%) in nuclear transport. The loop mutants tested have block sequence substitutions in one loop which alter the sequence but maintain the single-stranded secondary structure (56). Nucleotide substitutions that alter the 3′ sequence of loop C or the noncanonical base pairing in loop E (57, 72) resulted in a significant reduction in nuclear transport. The deletion of bulged nucleotides did not impair transport. In contrast, alteration of the hinge nucleotide at position 66 resulted in a 57% reduction in nuclear transport, suggesting this nucleotide is located in a key region of the 5S rRNA. This is in line with a previous study showing that nucleotides in the hinge region of the 5S rRNA, the junction of the three helical domains, play a central role in determining the coaxial stacking interactions and tertiary structure of the RNA and are critical for TFIIIA recognition (8). In summary, specific structural elements of the 5S rRNA molecule have been shown to be important for nuclear transport, but no single region of 5S rRNA is solely responsible.

TFIIIA has been implicated in mediating 5S rRNA nuclear export (31), but indirect evidence suggests that 7S RNPs are not import competent (3, 44). In order to determine whether there is a correlation between wild-type TFIIIA binding affinity and wild-type nuclear transport, results on nuclear transport of the mutants are compared with data on their ability to bind TFIIIA in vitro from previous studies (7, 8, 56, 74). In general, the two sets of data are comparable (Table 1); there are, however, important exceptions. For example, mutants 10–13, 57–62, and G109 possess low binding affinities for TFIIIA (17 to 40% of oocyte-type 5S rRNA), yet the measure of nuclear transport is closer to that of oocyte-type 5S rRNA (63 to 77%). Furthermore, other mutants (e.g., 96–101 and 14–15) with higher TFIIIA binding affinities (59 to 85%) exhibit defective nuclear transport (36 to 49%). Since some of the structural requirements of 5S rRNA for nuclear transport and 7S RNP assembly differ, this suggests that TFIIIA binding is not a prerequisite for nuclear targeting.

Nuclear import of 5S rRNA and mutant RNA molecules occurs by a mediated process. One possible explanation for oocyte-type and mutant 5S rRNAs entering the nucleus is that their small size allows for unrestricted entry. 5S rRNA is a 121-nucleotide molecule of approximately 41 kDa. The nuclear pore complex contains an aqueous channel of 9- to 11-nm diameter which acts like a molecular sieve; this channel allows rapid, nonselective diffusion of molecules of approximately 20 to 40 kDa, while excluding larger cytoplasmic proteins (52). A truncated, 98-nucleotide 5S rRNA does not enter the nucleus (Fig. 2) (3), however, which implies that nuclear uptake of full-length 5S rRNA occurs by a mechanism other than unrestricted entry. Further, comparing 5S rRNA and a globular protein may not be a valid comparison; the frictional ratio of naked 5S rRNA has been shown to be higher than that of 7S RNPs, suggesting that 5S rRNA is more elongated (17).

Translocation across the nuclear envelope occurs by an energy-dependent process (2, 51, 55). Thus, sensitivity to ATP depletion is one criterion for distinguishing active transport from diffusion. We therefore tested the effect of cytoplasmic ATP depletion on 5S rRNA nuclear import.

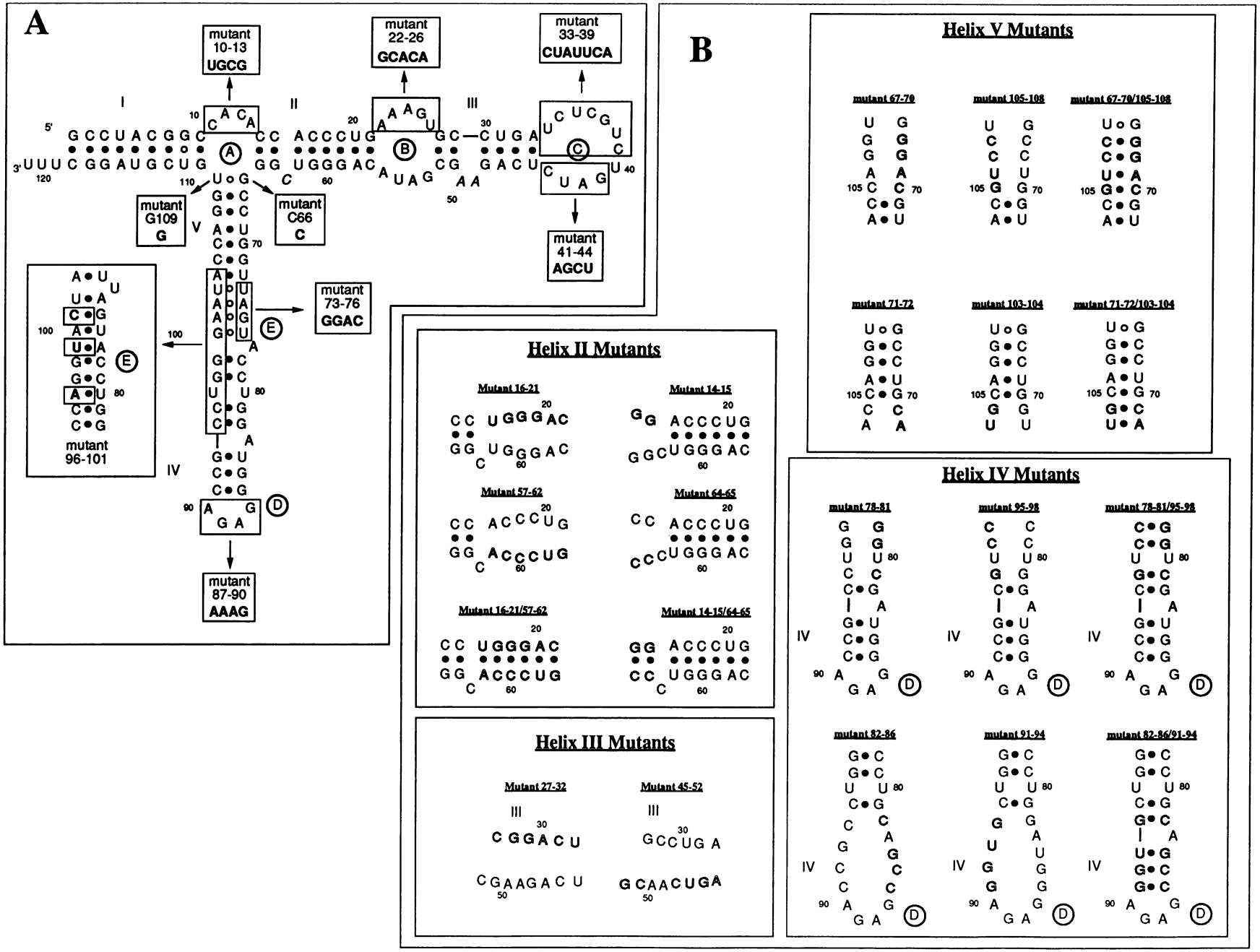

FIG. 1. Secondary structure of *X. laevis* oocyte-type 5S rRNA showing mutant nucleotide substitutions and deletions. (A) Single-strand substitution mutations. The bulged nucleotides deleted (A at nucleotides 49 and 50 and C at nucleotide 63) are indicated by italicized letters. (B) Helix mutants. Only the relevant region of the 5S rRNA is shown. Nucleotide substitutions are indicated by outlined letters.

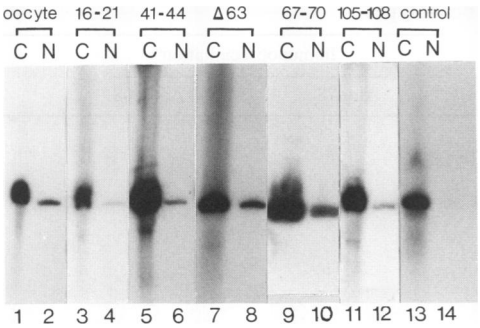

FIG. 2. Intracellular distribution of ^{32}P-labelled oocyte-type or mutant 5S rRNA species following microinjection in *X. laevis* oocytes. ^{32}P-labelled oocyte-type or mutant 5S rRNA was microinjected into the cytoplasm of fully grown oocytes. After overnight (18-h) incubation, RNA was extracted from five pooled nuclear (N) and cytoplasmic (C) fractions and separated electrophoretically on denaturing 8% polyacrylamide gels containing 8 M urea. The numbers refer to those nucleotides in the oocyte-type 5S rRNA (oocyte) which have been substituted or deleted (Fig. 1). A truncated 5S rRNA molecule consisting of nucleotides 1 to 98 (control) which does not enter the nucleus was included as a control for isolation of nuclei free of cytoplasmic contamination.

After injection of the ATP-hydrolyzing enzyme apyrase, ATP depletion was verified by a luciferase assay. At a final intracellular concentration of 100 U/ml, there was a rapid decline in ATP levels from 2 mM to 10 to 40 μM. This decrease occurred within 30 min and remained low after 25 h of incubation (data not shown). As shown in Fig. 3, ATP depletion significantly inhibited nuclear import of oocyte-type 5S rRNA (lane 4). The autoradiogram was deliberately overexposed to demonstrate the absence of detectable levels of labelled 5S rRNA in the nuclear fraction.

Another criterion for signal-mediated import via the nuclear pore complex is sensitivity to the lectin WGA. WGA is known to inhibit active transport by binding to *N*-acetylglucosamine (GlcNAc)-containing proteins present in the nuclear pore complex (25, 26, 65). Preinjection of WGA significantly inhibited nuclear import of oocyte-type 5S rRNA (Fig. 4A, lane 4). To verify that inhibition of import resulted from WGA interacting with GlcNAc-containing glycoproteins, WGA was coinjected with GlcNAc. Figure 4A (lane 6) shows that the presence of 50 mM GlcNAc abolished WGA-induced inhibition of import. The inhibitory effect of preinjected WGA on oocyte-type 5S rRNA was dose dependent (Fig. 4B), with 86% inhibition occurring at a concentration of 0.1 mg/ml. To ensure that WGA was not acting by physically occluding the nuclear pores, small fluorescently labelled dextrans (10 kDa) were microinjected into the oocyte cytoplasm. After overnight incubation, oocytes were dissected, and the nuclei were viewed by fluorescence microscopy. Preinjection of WGA had no effect on nonselective diffusion of small dextrans, and large fluorescently labelled dextrans (150 kDa) were excluded from the nucleus (data not shown). Similar results have been obtained in cultured somatic cells (15, 25).

An additional criterion for mediated transport is exclusion of nuclear proteins from the nucleus in chilled cells. Nuclear accumulation of large and small karyophilic proteins is inhibited by chilling, which slows enzyme-mediated reactions, whereas small proteins lacking nuclear localization signals may diffuse freely across the nuclear envelope, regardless of the temperature (10). Thus, 5S rRNA nuclear import was tested for temperature dependence. Figure 5 shows that the nuclear import of oocyte-type 5S rRNA was inhibited in chilled oocytes (lane 4). Chilling did not inhibit nonselective diffusion of a small fluorescent dextran into the nucleus (data not shown).

To determine whether nuclear import of mutant 5S rRNAs occurs by a mediated process or by nonselective diffusion, loop C mutant 41–44 and loop E mutant 96–101 were tested, since these RNAs have reduced abilities to enter the nucleus relative to that of oocyte-type 5S rRNA (Table 1). Preinjection of WGA significantly inhibited nuclear import of both mutant 5S rRNAs, although mutant 41–44 was less sensitive. Data are summarized in Table 2. Nuclear import of mutants 41–44 and 96–101 was also inhibited in chilled oocytes (Table 2), although both mutants were less sensitive than oocyte-type 5S rRNA. The possibility remains that nuclear entry of these mutants is partly diffusional; binding to an import receptor protein may be of low affinity or weakly temperature sensitive. Not all RNA-protein interactions, however, are temperature sensitive, because assembly of microinjected 5S rRNA into 7S RNPs (storage particles) does occur in chilled oocytes (data not shown).

In summary, nuclear import of 5S rRNA is sensitive to general inhibitors of nuclear pore-mediated translocation, so import is likely accomplished by a pathway similar to that used by karyophilic proteins.

5S rRNA is assembled into 60S ribosomal subunits in the nucleus. Although indirect evidence to date suggests that cytoplasmic 5S rRNA returns to the nucleus for ribosome incorporation (3, 18), a nuclear assembly site has not yet been demonstrated. To deduce the site of 5S rRNA assembly into 60S ribosomal subunits, labelled 5S rRNA was microinjected into the cytoplasm and subsequently immunoprecipitated from the nuclear versus cytoplasmic compartment.

The anti-60S ribosomal subunit serum detects two major bands in extracts of 60S ribosomal subunits (Fig. 6A and B, lanes 3); one band is approximately 37 kDa, and the other is approximately 14 to 15 kDa. The 37-kDa band corresponds to ribosomal protein L2; the 14- to 15-kDa band is not as easily identified but migrates similarly to ribosomal proteins in the range of L17 to L20 (53). This antiserum also reacts slightly with an approximately 18-kDa band in extracts of 40S ribosomal subunits (Fig. 6A and B, lanes 4). In the 7S fraction from a sucrose gradient, apart from the high-molecular-mass bands which probably represent polysaccharides, there is no cross-reaction (Fig. 6A and B, lanes 2). This antiserum does not immunoprecipitate 7S RNPs (Fig. 6C, lane 2). Intact ribosomes are immunoprecipitated by the anti-60S serum; the immunoprecipitates contain proteins that react with anti-40S ribosomal subunit serum as revealed by immunoblotting and contain both 28S and 18S rRNA as revealed by Northern blot (RNA) analysis (data not shown).

60S subunits containing labelled 5S rRNA were first faintly detectable in the nuclear fraction after 18 h but were not detectable in the cytoplasm (data not shown). After 48 h, labelled 5S rRNA was immunoprecipitated as 60S subunits from both the cytoplasmic fraction (Fig. 7, lane 2) and the nuclear fraction (lane 4), thus indicating a nuclear site of assembly, followed by export to the cytoplasm.

In a previous study, 7S RNPs were detected only in the oocyte cytoplasm after microinjection of labelled 5S rRNA and immunoprecipitation with anti-TFIIIA antibodies (3). Since TFIIIA has been shown to bind nuclear 5S rRNA (31), we repeated these assays with a different antibody preparation to determine whether a small nuclear pool of 7S RNPs was present. The antibodies raised against TFIIIA react with

TABLE 1. Summary of mutant phenotypes

Mutant designation[a]	Nuclear transport relative to oocytes type[b]	TFIIIA K_a in vitro[c]	Immunoprecipitation[d]		7S RNP EMSA[e]
			Anti-7S	Anti-60S	
Oocyte type	1.00	1.00	+	+	+
Helix II					
14–15 (HB)	0.49 ± 0.03 (3)	0.85 ± 0.22	IR	IR	+
64–65 (HB)	0.97 (1)	0.74 ± 0.24	−	+	+
14–15/64–65 (HM)	1.04 ± 0.16 (3)	1.11 ± 0.32	IR	+	+
16–21 (HB)	0.25 ± 0.05 (2)	0.32 ± 0.15	+	IR	ND
57–62 (HB)	0.73 (1)	0.40 ± 0.15	+	+	+
16–21/57–62 (HM)	1.88 (1)	1.09 ± 0.48	+	+	+
Helix III					
27–32 (HB)	0.70 ± 0.23 (2)	0.75 ± 0.10	+	+	+
45–52 (HB)	1.45 ± 0.34 (3)	0.76 ± 0.12	+	+	+
Helix IV					
78–81 (HB)	0.99 (1)	0.88 ± 0.01	+	+	ND
95–98 (HB)	0.86 (1)	0.78 ± 0.02	+	IR	+
78–81/95–98 (HM)	1.19 ± 0.46 (3)	0.86 ± 0.01	+	+	ND
82–86 (HB)	1.75 ± 0.02 (2)	0.81 ± 0.30	+	+	ND
91–94 (HB)	0.85 ± 0.31 (4)	0.96 ± 0.18	+	+	+
82–86/91–94 (HM)	1.38 ± 0.19 (2)	1.21 ± 0.35	+	+	ND
Helix V					
67–70 (HB)	1.18 ± 0.44 (2)	0.75 ± 0.12	−	+	+
105–108 (HB)	0.46 ± 0.15 (4)	0.39 ± 0.06	+	+	ND
67–70/105–108 (HM)	1.07 ± 0.34 (2)	0.71 ± 0.01	+	IR	+
71–72 (HB)	0.44 ± 0.12 (3)	0.35 ± 0.21	+	IR	ND
103–104 (HB)	0.48 ± 0.18 (3)	0.50 ± 0.23	+	IR	ND
71–72/103–104 (HM)	1.37 ± 0.43 (3)	1.18 ± 0.32	+	+	ND
Loop A					
10–13 (SEQ)	0.63 ± 0.00 (2)	0.30 ± 0.01	−	−	−
Loop B					
22–26 (SEQ)	1.18 (1)	1.00 ± 0.02	+	+	+
Loop C					
33–39 (SEQ)	0.97 ± 0.25 (3)	1.00 ± 0.02	+	+	+
41–44 (SEQ)	0.33 ± 0.06 (3)	0.40 ± 0.10	+	+	+
Loop D					
87–90 (SEQ)	1.26 (1)	0.71 ± 0.10	+	+	+
Loop E					
73–76 (SEQ)	1.60 ± 0.20 (2)	0.57 ± 0.02	−	+	+
96–101 (SEQ)	0.36 ± 0.04 (3)	0.59 ± 0.01	−	−	+
Bulged nucleotides					
Δ49,50 (DEL)	0.76 ± 0.38 (2)	1.00 ± 0.02	+	−	+
Δ63 (DEL)	1.04 ± 0.40 (4)	1.00 ± 0.02	+	−	+
Hinge nucleotides					
C66 (SEQ)	0.43 ± 0.05 (2)	0.12 ± 0.01	+	+	+
G109 (SEQ)	0.77 ± 0.02 (2)	0.17 ± 0.05	−	+	+

[a] The numbers in the designations refer to those nucleotides in 5S rRNA which have been substituted or deleted (Fig. 1). HB, helix breaking; HM, helix maintaining; SEQ, sequence mutation; DEL, deletion.

[b] Assays were performed as described in the legend to Fig. 2; nuclear transport was quantified by densitometry of suitable exposures of autoradiograms (within the linear range of signal intensity of the film), relative to the steady-state levels of oocyte-type 5S rRNA localized to the nucleus during each experiment. Experimental values are presented (mean ± standard deviation from the mean). Numbers in parentheses indicate the number of independent experiments performed.

[c] In vitro binding data is summarized from references 7, 8, 56, 58, and 74.

[d] Assays were performed as described in the legend to Fig. 8A. Protein binding was quantified by qualitative estimation of relative amounts of bound and free RNA. IR, inconclusive results caused by RNA with low specific activity.

[e] Electrophoretic mobility shift assays (EMSAs) were performed as described in the legend to Fig. 8C. ND, not determined.

the relevant protein (Fig. 6C, lane 1); no cross-reaction was noted with any other protein by immunoblotting (70). Under similar conditions, in this present study 7S RNPs containing labelled 5S rRNA were detectable only in the cytoplasmic fraction from 10 oocytes incubated for 21 h after microinjection (data not shown). However, a small nuclear pool of 7S RNPs was revealed by increasing the incubation time after microinjection to 48 h, increasing the specific activity of the labelled 5S rRNA, and increasing the sample size to 20 oocytes (Fig. 7, lane 3).

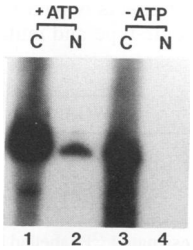

FIG. 3. Nuclear import of oocyte-type 5S rRNA is ATP dependent. Oocytes were preinjected with a final intracellular concentration of 100 U of apyrase per ml (−ATP), or PBS as a control (+ATP). After incubation for 30 min at 18°C, ^{32}P-labelled oocyte-type 5S rRNA was injected into the cytoplasm, and the oocytes were incubated for a further 6 h. The analysis for nuclear import was then performed as described in Fig. 2. C, cytoplasm; N, nucleus.

Sequence and structural requirements of 5S rRNA for 60S ribosomal subunit assembly and 7S RNP assembly in vivo. Since the results above demonstrated that the first requirement for subunit assembly is nuclear targeting, it was of interest to ascertain which sequence and structural elements of 5S rRNA are required for incorporation into 60S ribosomal subunits. Immunoprecipitation assays were thus performed on microinjected oocytes with antibodies against 60S subunits.

The results of several representative experiments are shown in Fig. 8A, and the complete data are presented in Table 1. Of 31 mutants, only 4 were completely defective in ribosome incorporation. Of these four mutants, two had mutations in single-strand loop regions, mutants 96–101 (Fig. 8A, lane 14) and 10–13 (lane 16), indicating that these regions of the molecule are important in recognition or binding of 5S rRNA with other ribosomal components. Comparable amounts of oocyte-type and mutant 5S rRNAs were detectable in the immunosupernatant fractions, demonstrating that the mutants were not degraded during the 48-h incubation (Fig. 8B). The simplest explanation for mutant 96–101 not being detected in ribosomes is that this results from impaired nuclear transport (Table 1). However, mutant 41–44 is equally defective in nuclear transport, yet it is found incorporated into 60S ribosomal subunits (lane 12). The other two defective mutants were Δ49,50 (lane 18) and Δ63 (lane 20), indicating that these bulged nucleotides are important for 5S rRNA incorporation into the 60S subunit. Results were inconclusive for six mutants (Table 1). The possibility that the absence of these labelled RNAs in 60S ribosomal subunits was due to the low specific activity of the RNA could not be ruled out. Despite repeated attempts to increase the specific activity, these particular mutant 5S rRNA gene constructs continued to provide poor templates for transcription.

In addition to assaying different mutant 5S rRNA molecules for their incorporation into 60S subunits, immunoprecipitation of 7S RNPs was also performed with anti-TFIIIA antibodies. This qualitative analysis provided a comparison with the in vitro data previously obtained for TFIIIA binding to the mutant 5S rRNA molecules (summarized in Table 1). All but six mutant 5S rRNAs were found to be able to bind TFIIIA in the oocyte in an immunodetectable complex. One of these defective mutants, 10–13 (Fig. 8A, lane 15), corresponds with the in vitro data; mutant 10–13 has a K_a of 0.30 and is 20 times less effective in inhibiting TFIIIA binding than oocyte-type 5S rRNA is (56). Negative results with other mutants, however, were contradictory. For example,

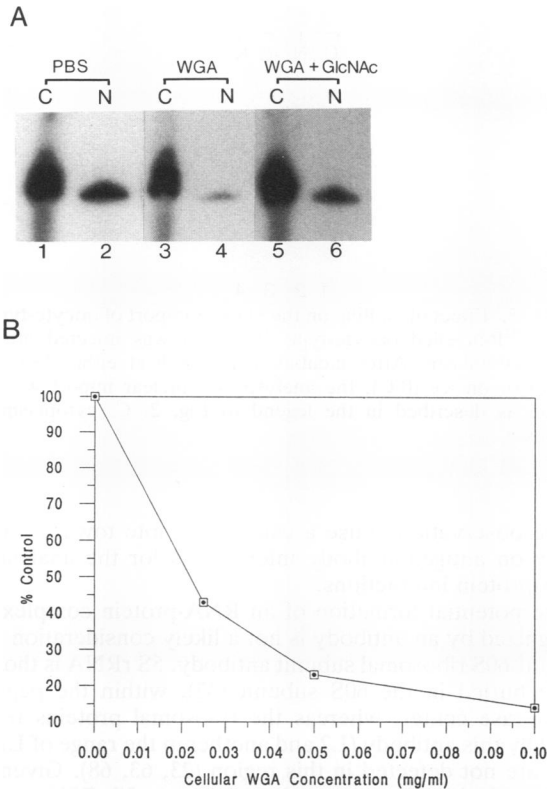

FIG. 4. Nuclear import of oocyte-type 5S rRNA is inhibited by WGA. (A) Oocytes were preinjected with WGA at a final intracellular concentration of 0.1 mg/ml or with WGA (0.1 mg/ml) and 50 mM *N*-acetylglucosamine (WGA + GlcNAc). PBS was preinjected as a control. After incubation for 3 h, ^{32}P-labelled oocyte-type 5S rRNA was injected into the cytoplasm and the oocytes were incubated for a further 18 h. The analysis for nuclear import was then performed as described in the legend to Fig. 2. C, cytoplasm; N, nucleus. (B) Dose-dependent inhibition of 5S rRNA nuclear import. Oocytes were preinjected with 50 nl of WGA at various concentrations. The final intracellular concentration of WGA is denoted on the *x* axis. The nuclear import of ^{32}P-labelled 5S rRNA was assayed 18 h after injection. Import in the presence of WGA is expressed as a percentage of the import of 5S rRNA in control oocytes preinjected with PBS only (% Control).

mutant 67–70 (Fig. 8A, lane 7) was not immunoprecipitated with anti-TFIIIA yet has an in vitro binding affinity of 0.75 (74). To clarify these results, homogenates of microinjected oocytes were analyzed by electrophoretic mobility shift assays. As shown in Fig. 8C, mutant 67–70 (lane 5) clearly showed a band shift present at a position corresponding to that of 7S RNPs. Similarly, mutants 64–65, 73–76, 96–101, and G109 which were not immunodetectable by anti-TFIIIA showed band shifts corresponding to 7S RNPs (Fig. 8A and C and Table 1). In contrast, consistent with the in vitro binding data and immunoprecipitation data, no band shift corresponding to 7S RNPs was detected with mutant 10–13 (Fig. 8C, lane 8). The data are summarized in Table 1.

The inconsistent anti-TFIIIA immunoprecipitation assay and electrophoretic mobility shift assay results described above can be explained by reasoning that for these mutant 5S rRNA molecules, the 5S rRNA-TFIIIA complexes that are formed have different conformations which are not recognized by the anti-TFIIIA antibody or that the complexes formed are not stable under the assay conditions.

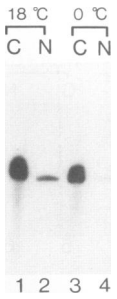

FIG. 5. Effect of chilling on the nuclear import of oocyte-type 5S rRNA. ^{32}P-labelled oocyte-type 5S rRNA was injected into the oocyte cytoplasm. After incubation for 18 h at either 18°C as a control or on ice (0°C), the analysis for nuclear import was performed as described in the legend to Fig. 2. C, cytoplasm; N, nucleus.

These observations raise a cautionary note toward relying solely on antigen-antibody interactions for the analysis of RNA-protein interactions.

The potential formation of an RNA-protein complex not recognized by an antibody is not a likely consideration with the anti-60S ribosomal subunit antibody. 5S rRNA is thought to be buried in the 60S subunit (42), within the peptidyl transferase center, whereas the ribosomal proteins recognized by this antibody (L2 and another in the range of L17 to L20) are not detected in this region (23, 63, 68). Given the apparent lack of direct cross-links between 5S rRNA and the ribosomal proteins recognized by the anti-60S antibody, it is unlikely that any conformational change resulting from incorporation of a mutant 5S rRNA molecule into the 60S subunit would be transmitted to these ribosomal proteins; thus, the recognition or binding of the anti-60S antibody to these epitopes would not be affected.

TABLE 2. Summary of effects of WGA and chilling on nuclear import of oocyte-type and mutant 5S rRNA

RNA	% Control[a]	
	WGA	0°C
Oocyte type	14.6 (±0.4)	4.1 (±2.1)
41–44	42.0 (±3.4)	43.0 (±3.0)
96–101	17.0 (±1.6)	22.0

[a] Nuclear import of the designated ^{32}P-labelled RNA (Fig. 1) was assayed in samples of five oocytes as described in the legend to Fig. 4A for WGA (final intracellular concentration of 0.2 mg/ml) and in the legend to Fig. 5 for chilling. Import was quantified by densitometry and expressed as a percentage (± standard deviation from the mean) of the amount of import in control oocytes (% Control). Tabulated results are from two samples, except for mutant 96–101 (0°C) for which one sample was used.

In summary, specific structural elements of the 5S rRNA molecule have been shown to be critical for ribosome incorporation. Deletion of bulged nucleotides and nucleotide substitutions that alter the sequence of loop A or the noncanonical base pairing in loop E (57, 72) abolish ribosome incorporation. Some of the structural elements of 5S rRNA required for ribosome incorporation differ from those elements required for nuclear transport. Further, some of the structural requirements of 5S rRNA for 7S RNP assembly and for 60S ribosomal subunit assembly differ.

DISCUSSION

From the 5S rRNA mutants studied in this report, a picture emerges of complex and nonidentical structural features within the central domain of the RNA molecule that

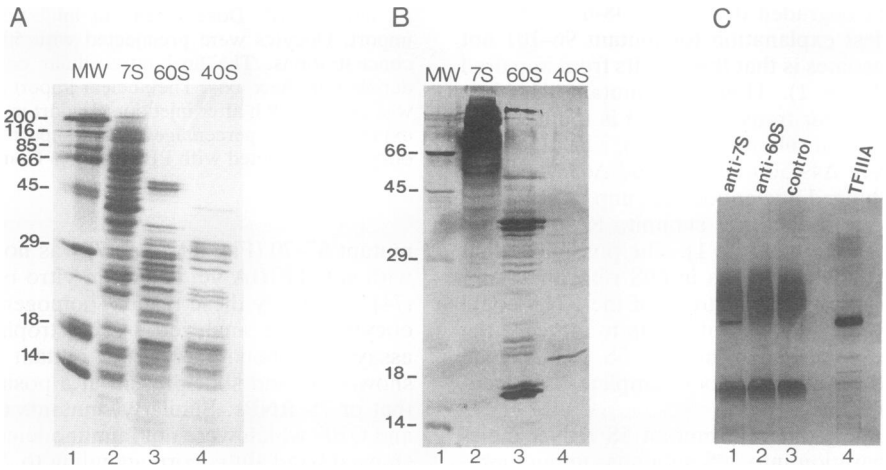

FIG. 6. Control of antiserum specificity by immunoblotting and immunoprecipitation assays. Total protein from 7S fractions and from ribosomal subunits purified from *Xenopus* ovaries was fractionated by electrophoresis in 15% polyacrylamide gels containing 0.1% sodium dodecyl sulfate (SDS) and either stained with Coomassie blue (A) or transferred to Immobilon-P polyvinylidene difluoride membranes and probed with anti-60S ribosomal subunit antiserum (B). The antigen-antibody complexes were revealed by treatment with ^{35}S-labelled protein A and then autoradiography (48 h). The high-molecular-mass bands in lane 2 of panel B are probably polysaccharides. Lanes: MW, protein molecular mass markers (in kilodaltons); 7S, approximately 10 μg of protein purified from the 7S region of a sucrose gradient; 60S, approximately 3 μg of protein from 60S ribosomal subunits purified by sucrose gradient centrifugation; 40S, approximately 1 μg of protein from 40S ribosomal subunits purified by sucrose gradient centrifugation. (C) 7S RNPs purified from immature *Xenopus* ovaries were incubated with protein A-Sepharose-antibody complexes in an immunoprecipitation assay. Proteins were recovered and separated electrophoretically on 12% polyacrylamide gels containing 0.1% SDS and then silver stained. Lanes: anti-7S, immunoprecipitation with anti-TFIIIA; anti-60S, immunoprecipitation with anti-60S ribosomal subunit; control, mock immunoprecipitation with anti-60S antiserum, but without 7S RNPs; TFIIIA, TFIIIA protein from 7S RNPs.

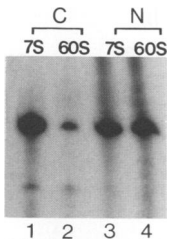

FIG. 7. Intracellular localization of microinjected 5S rRNA in 60S ribosomal subunits and 7S RNPs. Labelled 5S rRNA was microinjected into the cytoplasm of oocytes. After incubation for 48 h, 20 oocytes were manually fractionated into cytoplasmic (C) and nuclear (N) compartments. Homogenates from each compartment were incubated with protein A-Sepharose-antibody complexes in an immunoprecipitation assay. Labelled RNAs were recovered and separated electrophoretically on 8% polyacrylamide gels containing 8 M urea. Lanes: 7S, immunoprecipitation with anti-TFIIIA; 60S, immunoprecipitation with anti-60S ribosomal subunit.

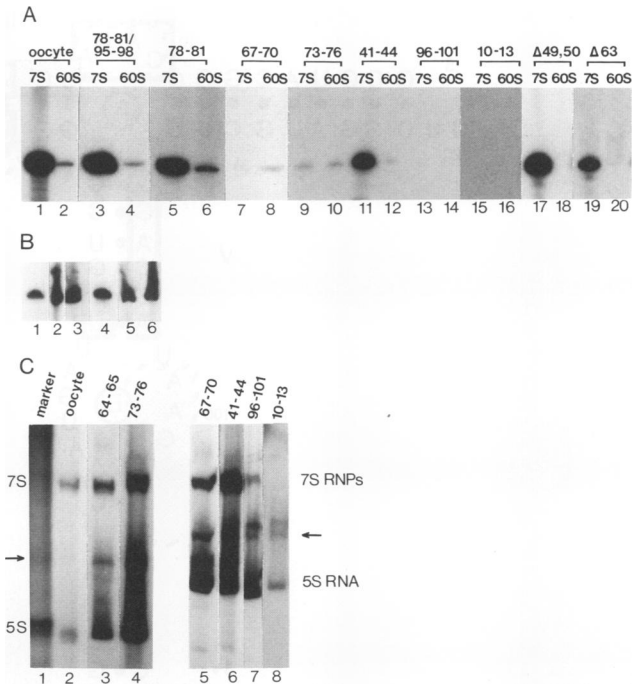

FIG. 8. Assembly of mutant 5S rRNAs into 60S ribosomal subunits and 7S RNPs. (A) ^{32}P-labelled oocyte-type or mutant 5S rRNA was microinjected into the oocyte cytoplasm. After 48 h, homogenates of whole oocytes were analyzed by immunoprecipitation assay as described in the legend to Fig. 7. Labelled RNAs were recovered and separated electrophoretically on 8% polyacrylamide gels containing 8 M urea. The numbers in the mutant designations refer to those nucleotides in oocyte-type 5S rRNA (oocyte) which have been substituted or deleted (Fig. 1). Lanes: 7S, immunoprecipitation with anti-TFIIIA; 60S, immunoprecipitation with anti-60S ribosomal subunit. (B) Labelled RNAs recovered from immunosupernatant fractions, from the anti-60S immunoprecipitation assays in panel A. Lane 1, oocyte-type RNA; lane 2, mutant 67–70; lane 3, mutant 96–101; lane 4, mutant 10–13; lane 5, mutant Δ49,50; lane 6, mutant Δ63. (C) Electrophoretic gel mobility shift assays of mutant 5S rRNAs. Labelled oocyte-type or mutant 5S rRNA was injected into the oocyte cytoplasm. After 48 h, homogenates were electrophoresed on nondenaturing 6% polyacrylamide gels containing 0.1% Triton X-100. The location of 7S RNPs was determined by running an additional lane of unlabelled, purified 7S RNPs; this lane was stained with ethidium bromide and visualized by UV illumination (not shown). The arrows indicate a band shift that may represent the 5S rRNA-ribosomal protein L5 complex, but this has yet to be confirmed. Marker, ^{32}P-labelled 5S rRNA. Lanes 1 to 4 and lanes 5 to 8 represent experiments in which samples were electrophoresed for different lengths of time.

are required for nuclear transport, assembly into 7S RNPs (storage particles) and assembly into 60S ribosomal subunits (Fig. 9). 5S rRNA is assembled into 60S ribosomal subunits in the nucleus; thus, the first requirement for subunit assembly is nuclear targeting. However, some of the structural elements of 5S rRNA required for ribosome incorporation differ from those elements required for nuclear transport (Fig. 9). Differences in the requirements for nuclear transport and TFIIIA binding suggest that TFIIIA binding is not a prerequisite for nuclear targeting of 5S rRNA.

Subcellular trafficking of 5S rRNA. Since 5S rRNA can be easily and specifically dissociated from intact ribosomes or subunits as a 5S rRNA-L5 complex (3), it was thought that the 5S rRNA might be added as a surface component to partially assembled subunits, hence a cytoplasmic site of integration of stored 5S rRNA seemed plausible. However, results presented here indicate that cytoplasmically stored 5S rRNA returns to the nucleus for assembly into the large 60S subunit. This conclusion is consistent with a recent study in *Saccharomyces cerevisiae*, which suggests that 5S rRNA is crucial to an early step in subunit assembly (69). Thus, analysis of intracellular distribution reflects the steady-state levels of 5S rRNA molecules reached after overnight incubation (3); 5S rRNA is released from storage, migrates into the nucleus, is assembled into ribosomes, and then is exported to the cytoplasm. Since ribosome assembly continues for a protracted period of time in *Xenopus* oocytes, only a small fraction of microinjected 5S rRNA would be expected to appear in the nucleus at any given time or be incorporated into nascent ribosomes. The latter is reflected in the small fraction of labelled 5S rRNA found in 60S subunits compared with 7S RNPs (storage particles). Interestingly, a number of mutants accumulated in the nucleus to a greater degree than oocyte-type 5S rRNA, for example, nuclear accumulation of mutant 16–21/57–62 was nearly twice that of oocyte-type 5S rRNA. It is not clear whether this distribution represents enhanced nuclear import or whether the increased nuclear accumulation of some mutants is due to a defect in another function, such as nuclear export.

Mediated nuclear import of 5S rRNA. Nuclear import of 5S rRNA is inhibited by cytoplasmic ATP depletion, WGA, and chilling. Similarly, nuclear protein import (2, 51, 55), export of ribosomal subunits (6), and mRNA export (16) require metabolic energy in vivo. The concentration of WGA required to inhibit 5S rRNA nuclear import is comparable to the concentration of WGA shown to inhibit the nuclear import of U6 small nuclear RNA, but 20 times less than the concentration required to inhibit nuclear import of U1 small nuclear RNA to a similar degree (see Fig. 3 in reference 48). These results correlate with studies on nuclear export of 5S rRNA and 5S rRNA-containing RNPs. Approximately 60% inhibition of ribosomal subunit export can be achieved when WGA is injected into the nucleus at a final concentration of 0.5 mg/ml (6), and export of 5S rRNA newly synthesized from microinjected cloned genes is significantly inhibited by preinjection of RL1, an antinucleoporin monoclonal antibody (24). The sensitivity of 5S rRNA to general inhibitors of nuclear transport suggests that 5S rRNA is targeted to oocyte nuclei by a receptor-mediated process.

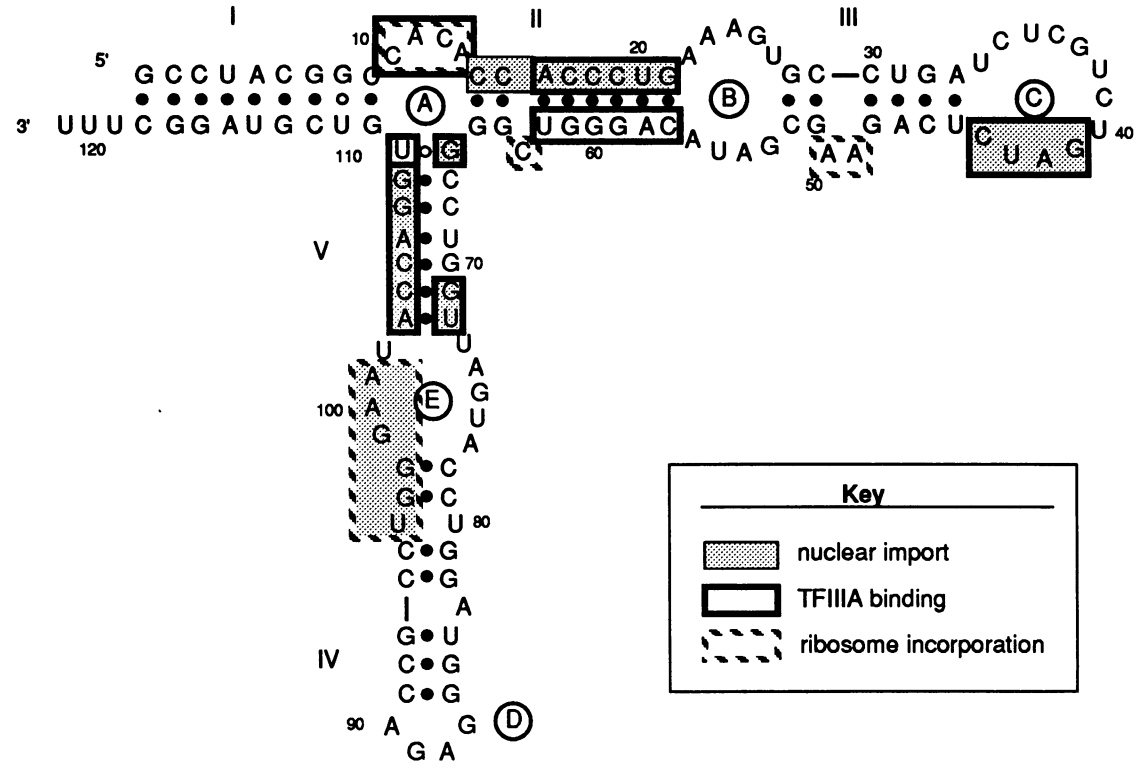

FIG. 9. Complex, nonidentical regions of 5S rRNA required for different functions. Shaded boxes indicate regions in which nucleotide substitution results in at least a 50% reduction in nuclear transport of 5S rRNA. Boxes outlined in bold indicate regions in which nucleotide substitution results in at least a 50% reduction in TFIIIA binding affinity. Boxes outlined in dashed lines indicate regions in which nucleotide substitution abolishes incorporation of 5S rRNA into the 60S ribosomal subunit.

Given the specificity of receptor-mediated processes, it seemed surprising that all mutants were capable of at least some degree of nuclear import. However, the results presented here are similar to an analysis of the nuclear export phenotypes of 30 different point mutants of human $tRNA_i^{Met}$ which revealed variable defects in transport; the percentage of microinjected tRNA exported to the cytoplasm ranged from 85 (wild type) to 34% (D stem mutant) (67). Glyceraldehyde-3-phosphate dehydrogenase binds two defective mutants with lower affinity than that of wild-type tRNA, suggesting that this protein may be involved in tRNA export (62). This comparison with tRNA export and a model for the tertiary structure of 5S rRNA provide some insight into 5S rRNA import. 5S rRNA has been proposed to adopt a Y-shaped structure in which the three helical domains are independent (13, 71), that is, the effect that a mutation has on the RNA structure is primarily restricted to the mutated loop (12, 40). If a protein involved in nuclear targeting makes numerous contacts with 5S rRNA, disruption of one small region may not disrupt binding in other regions, therefore potentially providing a stable complex that is still recognized by the transport machinery. Relatively small changes in complex stability may be masked at the saturating levels of RNA used in these experiments.

Guddat et al. (31) proposed that 5S rRNA nuclear export is mediated by either TFIIIA or L5 on the basis of studies showing that mutant RNA molecules that do not form immunodetectable complexes with these proteins are retained in the nucleus. Results of this present study suggest that TFIIIA binding is not a prerequisite for nuclear import of 5S rRNA, consistent with earlier work suggesting that 7S RNP transport is unidirectional (3, 44). A small nuclear pool of 7S RNPs containing labelled 5S rRNA was detected after microinjection of labelled 5S rRNA into the oocyte cytoplasm, but it is likely that these 7S RNPs were assembled after nuclear entry. If 5S rRNA enters the nucleus in excess of the amount required for assembly into nascent 60S ribosomal subunits, it may become associated with TFIIIA. Along these lines, it has been proposed that excess 5S rRNA may be targeted to the cytoplasm of mammalian somatic cells for degradation bound to a TFIIIA-like protein (39).

Ribosomal protein L5 and 5S rRNA form a stable complex prior to assembly of ribosomal subunits (3, 11, 64, 73). Cytoplasmic exchange between TFIIIA and L5 for binding of 5S rRNA correlates with the mobilization of 5S rRNA from storage within the *Xenopus* oocyte cytoplasm (3), suggesting that L5 may mediate 5S rRNA nuclear targeting. Little is known about the sequence and structural requirements for L5 binding to 5S rRNA, although chemical protection assays (35) and immunoprecipitation assays (3) indicate a binding domain similar to that of TFIIIA. Delineation of the sequence and structural requirements of 5S rRNA for L5 binding and correlation with data on subcellular localization should provide further insight into the role of L5 in nuclear import and nucleolar targeting of 5S rRNA. The possibility remains that other proteins, such as carriers that shuttle between the cytoplasm and the nucleus (9, 36, 46, 60), are involved in the subcellular trafficking of 5S rRNA.

5S rRNA structural elements required for ribosome assembly. Loop structures, bulged nucleotides, and non-Watson-

Crick base pairs of RNA have been shown to be of importance for specific protein recognition (4, 14, 54). Similarly, results presented here show that incorporation of 5S rRNA into ribosomes is abolished by deletion of bulged nucleotides, A at nucleotides 49 and 50 or C at nucleotide 63, and by nucleotide substitutions that alter the sequence of loop A or the noncanonical base pairing in loop E. These results correlate with results from other workers investigating prokaryotic 5S rRNA-protein interactions. *Escherichia coli* 5S rRNA has a structure similar to that of eukaryotic 5S rRNA, but *E. coli* 5S rRNA binds to three ribosomal proteins, L5, L18, and L25, rather than to one protein as in eukaryotes. A bulged nucleotide at position 65 in *E. coli* 5S rRNA, corresponding to position 63 in eukaryotic 5S rRNA, is necessary for binding of L18 to the 5S rRNA (reviewed in reference 29).

Various regions of 5S rRNA have been postulated to be of importance for ribosome assembly and function (69; for a review, see reference 29). Complementary base pairing between nucleotide sequences at the 5′ and 3′ ends of mouse 18S rRNA contained in the 40S ribosomal subunit and nucleotides 9 to 26 and 90 to 107 of 5S rRNA occurs in vitro (59). The functional significance of this stable interaction has yet to be determined. Interestingly, mutant 96–101 which has increased Watson-Crick base pairing in the loop E-helix IV region of 5S rRNA and mutant 10–13 which has an altered sequence in loop A were shown here to be defective in ribosome incorporation. These results indicate a correlation between the structural elements of the 5S rRNA molecule required for 60S ribosomal subunit assembly and the structural elements previously proposed to aid in formation of the 80S ribosome by 5S rRNA-nucleic acid interactions. Protein recognition of the noncanonical base pairing in loop E encompassing nucleotides 96 to 101 of the 5S rRNA molecule (57, 72) may be of importance for 60S ribosomal subunit assembly, as well as RNA-RNA hybridization.

5S rRNA represents an important model system for study of the regulated subcellular trafficking of RNA, because it involves many different components: shuttling of the 5S rRNA molecule across the nuclear envelope, a variety of RNA-protein interactions and exchanges, cytoplasmic localization of the 5S rRNA in RNPs (storage particles), and nucleolar targeting. Continued study of the effects of mutations by an in vitro binding assay (7, 8, 56, 74) and by the in vivo functional assays described here should help to further identify and clarify those sequence and structural elements of 5S rRNA required for some of its biological activities.

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