MOLECULAR AND CELLULAR BIOLOGY, Dec. 1989, p. 5281–5288
0270-7306/89/125281-08$02.00/0

Vol. 9, No. 12

Developmental Expression and 5S rRNA-Binding Activity of *Xenopus laevis* Ribosomal Protein L5

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Received 7 July 1989/Accepted 23 August 1989

Ribosomal protein L5 binds specifically to 5S rRNA to form a complex that is a precursor to 60S subunit assembly in vivo. Analyses in yeast cells, mammalian cells, and *Xenopus* embryos have shown that the accumulation of L5 is not coordinated with the expression of other ribosomal proteins. In this study, the primary structure and developmental expression of *Xenopus* ribosomal protein L5 were examined to determine the basis for its distinct regulation. These analyses showed that L5 expression could either coincide with 5S rRNA synthesis and ribosome assembly or be controlled independently of these events at different stages of *Xenopus* development. L5 synthesis during oogenesis was uncoupled from the accumulation of 5S rRNA but coincided with subunit assembly. In early embryos, the inefficient translation of L5 mRNA resulted in the accumulation of a stable L5-5S rRNA complex before ribosome assembly at later stages of development. Additional results demonstrated that L5 protein synthesized in vitro bound specifically to 5S rRNA.

A central problem of eucaryotic ribosome biosynthesis is identification of the regulatory pathways that direct gene products transcribed by three discrete RNA polymerases and synthesized in different cellular locations to be present in equimolar amounts in fully assembled subunits. A paradoxical feature of ribosome biogenesis during *Xenopus* development is that synthesis of individual ribosomal constituents is uncoupled from subunit assembly at distinct stages of both oogenesis and embryogenesis (reviewed in reference 43). For example, previtellogenic oocytes sequester 5S rRNA in a cytoplasmic 7S particle containing transcription factor TFIIIA for months before the onset of ribosome assembly during later stages of oogenesis (32, 34). During embryogenesis, the transcriptional activation of rRNA genes precedes the assembly of new ribosomes in the swimming tadpole (6, 7). Likewise, zygotic ribosomal protein mRNAs begin to accumulate after the midblastula transition even though they are excluded from polysomes until the tailbud stage (3, 36). The analysis of anucleolate embryos has revealed that both the onset of 5S rRNA synthesis and the translational activation of ribosomal protein mRNAs are independent of 18S and 28S rRNA synthesis (29, 35). These aspects of ribosome biosynthesis during *Xenopus* development differ from the balanced expression and synchronous assembly of the majority of ribosomal components in bacterial, yeast, and somatic mammalian cells (reviewed in reference 26).

Additional studies have indicated, however, that the expression of ribosomal protein L5 is an exception to the equimolar accumulation of eucaryotic ribosomal proteins. Expression of the homologous yeast ribosomal protein, YL3, is not coordinated with synthesis of other ribosomal proteins (14). An unusually large non-subunit-associated pool of L5 is present in mammalian cells in the form of an L5-5S rRNA complex that is a precursor to 60S subunit assembly (23, 33, 40). During *Xenopus* embryogenesis, newly synthesized L5 protein is first detected after the midblastula transition, which coincides with the onset of 5S rRNA gene transcription but precedes ribosome assembly (3, 36, 44).

In this study, the primary structure and developmental expression of *Xenopus* ribosomal protein L5 were examined to determine the basis for the distinct regulation of the protein. The results show that L5 mRNA is subject to the same translational control as are other ribosomal protein mRNAs during oogenesis and embryogenesis. L5 synthesis during oogenesis is uncoupled from the accumulation of 5S rRNA but coincides with subunit assembly. In contrast, the inefficient translation of L5 mRNA in early embryos results in the accumulation of a stable L5-5S rRNA complex before ribosome assembly at later stages of development. Additional results also demonstrate that L5 protein synthesized in vitro binds to 5S rRNA.

MATERIALS AND METHODS

Biological materials. Frogs were purchased from Xenopus I (Ann Arbor, Mich.). Oocytes were obtained by manual dissection of collagenase-disaggregated ovarian fragments and maintained in modified Barth saline (15) at 18°C. Oocytes were staged as described by Dumont (11). Maturation was induced in vitro with progesterone as described previously (19). Synchronously developing embryos were obtained by in vitro fertilization of eggs stripped from ovulating females. Embryonic stages refer to the normal table of Nieuwkoop and Faber (31).

Screening of a cDNA library, subcloning, and sequence determination of L5 cDNAs. A stage 17 embryonic cDNA library constructed in λgt10 (20) was probed by using a ^{32}P-labeled L5 cDNA insert from pL5-6-4 (10). Replica filters containing approximately 40,000 plaques were hybridized and washed under the high-stringency conditions described by Maniatis et al. (27). Positive hybridizing bacteriophage were purified, and inserts were analyzed by restriction endonuclease digestion and Southern blot hybridization to pL5-6-4. Purified phage cDNA inserts were isolated by partial digestion with *Eco*RI and subcloned into pSP65AT (2). DNA sequences were obtained by the dideoxy-chain termination procedure of Sanger et al. (39), using modified T7 DNA polymerase (Sequenase; U.S. Biochemical Corp., Cleveland, Ohio). Overlapping sequences for both strands were obtained by using synthetic oligonucleotide primers. DNA and protein sequence analyses were performed by using The DNA Inspector IIe (Textco).

Isolation of RNA and transcript analysis. Total RNA was

isolated as described previously (42). RNA was isolated from polysomal and nonpolysomal fractions prepared as described in detail by Baum et al. (2). RNAs were fractionated in denaturing formaldehyde-agarose gels and transferred to Nitroplus 2000 membranes (Micron Separations Inc.) as described previously (3, 42). To detect L5 mRNA, the L5b cDNA insert was cloned in the antisense orientation in pSP65. A ^{32}P-labeled RNA probe was synthesized by in vitro transcription of this template linearized with *Hin*dIII, using SP6 RNA polymerase as described elsewhere (28). L1 and translation elongation factor 1α (EF-1α) mRNAs were detected by using ^{32}P-labeled antisense RNAs derived from αL1-1.3 (42) and pG1 (21), respectively. S22 mRNA was detected by using a ^{32}P-labeled RNA probe to be described in detail elsewhere (B. D. Keiper and W. M. Wormington, manuscript in preparation).

Isolation of RNA for binding studies. Total RNA was extracted from previtellogenic stage I-II oocytes as described previously (42). RNA was 3′ end labeled with [5′-^{32}P]cytidine 3′,5′-bisphosphate (3,000 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.), using T4 RNA ligase (12). Unlabeled 5S rRNA was purified by 8% polyacrylamide–8 M urea gel electrophoresis of total immature oocyte RNA. The 5S rRNA was eluted and renatured as described by Huber and Wool (16).

Preparation of embryonic extracts and immunoprecipitations. Embryonic proteins were metabolically labeled by microinjection of 50 nl of 88 mM NaCl containing 5 μCi of [^{35}S]methionine (>1,000 Ci/mmol; Dupont, NEN) into the blastocoel of stage 8 or stage 11 embryos. Microinjected embryos were allowed to develop for an additional 6 or 12 h and frozen at −70°C. RNAs were metabolically labeled by microinjection of 50 nl of 88 mM NaCl containing 250 μCi of [α-^{32}P]UTP (>800 Ci/mmol; Dupont, NEN) into fertilized eggs before completion of the first cleavage division. Microinjected embryos were allowed to develop to stage 11 and were frozen at −70°C. Extracts for immunoprecipitation were prepared essentially as described in detail elsewhere (24, 38), with the following modifications. All steps were at 4°C. Ten labeled embryos were homogenized in 500 μl of NET-2 (50 mM Tris hydrochloride [pH 7.4], 150 mM NaCl, 0.05% [vol/vol] Nonidet P-40 5 μg of leupeptin per ml, 1 mM phenylmethylsulfonyl fluoride 2 mM dithiothreitol, 500 U of RNasin [Promega Biotec, Madison, Wis.] per ml). Homogenates were centrifuged at 10,000 × *g* for 5 min to pellet yolk platelets and pigment granules. The supernatant equivalent to two embryos was precleared by incubation with normal human serum and protein A-Sepharose before immunoprecipitation with anti-5SRNP serum (40) prebound to protein A-Sepharose. RNA was extracted from ^{32}P-labeled immunoprecipitates as described previously (24) and analyzed by 8% polyacrylamide–8 M urea gel electrophoresis. RNAs were visualized by autoradiography of dried gels. Proteins were extracted from ^{35}S-labeled immunoprecipitates with sodium dodecyl sulfate (SDS) sample buffer (22) and analyzed by 15% SDS-polyacrylamide gel electrophoresis. Gels were fixed, treated with Amplify (Amersham Corp., Arlington Heights, Ill.), dried, and exposed for autoradiography at −70°C.

In vitro translation and immunoprecipitation. In vitro translation reaction mixtures (50 μl) using wheat germ extract (Promega Biotec) contained 0.5 μg of SP6-L5 or SP6-L1 mRNAs and 50 μCi of [^{35}S]methionine (>1,000 Ci/mmol; Dupont, NEN); reaction mixtures were incubated at 25°C for 1 h. ^{35}S-labeled translation products were analyzed by 15% SDS-polyacrylamide gel electrophoresis as described above. For immunoprecipitations, 10^6 cpm of ^{32}P-labeled immature oocyte RNA (0.1 μg) and 5 μg of either unlabeled yeast tRNA or *Xenopus* 5S rRNA as a competitor were added to the translation reaction mixtures, and incubations were continued for an additional 30 min at 25°C. Reaction mixtures were diluted with NET-2 to a final volume of 500 μl, precleared by incubation with normal human serum and protein A-Sepharose, and immunoprecipitated with anti-5SRNP serum. RNAs were extracted and analyzed by 8% polyacrylamide–8 M urea gel electrophoresis as described above.

Gel retardation assays. Wheat germ extract translation reactions in 50-μl mixtures were performed as described above. Reaction mixtures were diluted with an equal volume of storage buffer (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES]-KOH [pH 7.6], 50 mM potassium acetate 2 mM magnesium acetate, 2 mM dithiothreitol, 5 μg of leupeptin per ml, 0.1% [vol/vol] Nonidet P-40, 10% [vol/vol] glycerol, 1,000 U of RNasin per ml). Unincorporated [^{35}S]methionine was removed by centrifugation of the diluted translation reaction mixtures through 1-ml Sephadex G-50 columns preequilibrated with storage buffer. Ribosomes and the small proportion (<25%) of ^{35}S-labeled L5 protein bound to endogenous 5S rRNA present in the wheat germ extract were removed from the G-50 eluate by centrifugation at 315,000 × *g* for 2 h at 4°C in a TLA-100.1 rotor (Beckman Instruments, Inc., Fullerton, Calif.). The ^{35}S-labeled L5 protein present in the supernatant retained its 5S rRNA-binding activity for at least 2 months when stored at −70°C. RNA binding was performed in 10-μl reaction mixtures containing 5 μl of ^{35}S-labeled postribosomal supernatant, 0.1 μg of 5S rRNA, 5 μg of yeast tRNA, 20 mM HEPES-KOH (pH 7.6), 50 mM potassium acetate 2 mM magnesium acetate 2 mM dithiothreitol, 0.5 mM spermidine, 2.5 μg of leupeptin per ml 0.05% (vol/vol) Nonidet P-40, 2.5% (vol/vol) glycerol, and 500 U of RNasin per ml. Reaction mixtures were incubated at 25°C for 30 min. Reactions were terminated by addition of 1 μl of 50% glycerol containing 0.3 mg each of xylene cyanol FF and bromophenol blue per ml. Complexes were electrophoresed at 100 V for 5 h at room temperature on nondenaturing 8% polyacrylamide gels in 1× TBE buffer (17). Gels were fixed with acetic acid-methanol, treated with Amplify (Amersham), dried, and exposed for autoradiography at −70°C.

RESULTS

Isolation and characterization of cDNAs encoding *Xenopus* ribosomal protein L5. The rat L5 cDNA insert from pL5-6-4 (10) was used as a probe to screen approximately 40,000 recombinants from a *Xenopus* stage 17 embryonic cDNA library constructed in λgt10 (20). Four clones were isolated and shown to contain overlapping inserts, as determined by restriction endonuclease and Southern blot analyses (data not shown). Two clones, designated L5a and L5b, contained inserts of sufficient length predicted to contain the entire L5-coding region. These two cDNA inserts were subcloned into pSP65AT (2), and overlapping DNA sequences were obtained for both strands.

The DNA sequences and derived amino acid sequences for L5a and L5b are shown in Fig. 1. Each cDNA contains a single open reading frame of 888 nucleotides encoding a protein of 296 amino acids. Neither cDNA, however, appears to be full length. Both the L5a and L5b cDNAs lack an extensive 3′-terminal poly(A) tract and the consensus polyadenylation sequence, AATAAA, in their 3′ untranslated

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                                                                                                met gly phe val lys
L5a  gaattccgGCGCCTTAACGTTAAAGGGCTGTGACTATCCGGTCCTAGCCCCGACGGGACAAAATAGAGCA ATG GGG TTC GTA AAG  77
L5b            gaattccgGTTAAAGGGCTGTGACTCTCCGGTCCTAGCCCCGACGGCTCAAAATAGAGCA ATG GGG TTC GTA AAG
                                                                                                met gly phe val lys   5

val val lys asn lys ala tyr phe lys arg tyr gln val lys phe arg arg arg arg glu gly lys thr asp
GTC GTC AAG AAC AAG GCT TAC TTT AAA AGG TAC CAG GTC AAG TTC CGC AGA AGG AGA GAG GGT AAG ACC GAT 149
GTC GTC AAG AAC AAG GCT TAT TTT AAG AGG TAC CAG GTC AAG TTC CGC AGA AGG AGA GAG GGC AAG ACC GAT
val val lys asn lys ala tyr phe lys arg tyr gln val lys phe arg arg arg arg glu gly lys thr asp  29

tyr tyr ala arg lys arg leu val ile gln asp lys asn lys tyr asn thr pro lys tyr arg met ile val
TAC TAT GCT CGC AAG CGA TTG GTG ATC CAG GAT AAG AAC AAG TAC AAT ACT CCC AAG TAC AGG ATG ATT GTA 221
TAC TAT GCT CGC AAG CGA CTG GTG ATC CAG GAT AAG AAC AAG TAC AAT ACT CCC AAG TAC AGG ATG ATT GTA
tyr tyr ala arg lys arg leu val ile gln asp lys asn lys tyr asn thr pro lys tyr arg met ile val  53

arg val thr asn arg asp ile ile cys gln ile ala tyr ala arg ile glu gly asp met ile val cys ala
CGT GTT ACC AAC AGA GAC ATC ATC TGC CAG ATT GCT TAT GCT AGG ATC GAG GGA GAT ATG ATT GTT TGC GCT 293
CGT GTC ACC AAC AGA GAC ATC ATC TGC CAG ATT GCT TAT GCT AGG ATT GAG GGA GAC ATG ATT GTA TGT GCT
arg val thr asn arg asp ile ile cys gln ile ala tyr ala arg ile glu gly asp met ile val cys ala  77

ala tyr ala his glu leu pro lys tyr gly ile lys val gly leu thr asn tyr ala ala ala tyr cys thr
GCC TAT GCT CAT GAG CTT CCC AAG TAT GGT ATC AAA GTT GGG CTG ACA AAC TAC GCT GCA GCA TAT TGT ACT 365
GCT TAT GCT CAT GAG CTT CCC AAG TAT GGT GTC AAA GTT GGG CTG ACA AAC TAT GCT GCA GCA TAT TGT ACT
ala tyr ala his glu leu pro lys tyr gly val lys val gly leu thr asn tyr ala ala ala tyr cys thr 101

gly leu leu leu ala arg arg leu leu asn lys phe gly leu asp lys val tyr glu gly gln val glu val
GGC CTG CTG CTG GCT CGT AGG CTT CTC AAC AAA TTC GGT CTG GAC AAA GTT TAC GAA GGC CAG GTA GAA GTA 437
GGC CTG CTG CTG GCT CGT AGG CTT CTC AAC AAA TTC GGT CTG GAC AAA GTA TAC GAA GGT CAA GTG GAA GTA
gly leu leu leu ala arg arg leu leu asn lys phe gly leu asp lys val tyr glu gly gln val glu val 125

thr gly asp glu tyr asn val glu ser ile asp gly glu pro gly ala phe thr cys tyr leu asp ala gly
ACT GGA GAT GAG TAC AAT GTA GAG AGC ATT GAT GGG GAA CCT GGT GCC TTC ACA TGC TAC CTT GAT GCC GGC 509
ACA GGA GAT GAG TAC AAT GTA GAG AGC GTT GAT GGT GAA CCT GGT GCC TTT ACA TGC TAC CTT GAT GCC GGC
thr gly asp glu tyr asn val glu ser val asp gly glu pro gly ala phe thr cys tyr leu asp ala gly 149

leu thr arg thr thr thr gly asn lys val phe gly ala leu lys gly ala val asp gly gly leu ser ile
CTG ACC AGA ACT ACC ACT GGA AAC AAA GTT TTT GGT GCT CTT AAG GGA GCT GTG GAT GGA GGT TTA TCT ATT 581
CTC ACC AGA ACC ACC ACT GGA AAC AAA GTT TTT GGT GCT CTT AAG GGA GCT GTT GAT GGA GGT TTA TCT ATT
leu thr arg thr thr thr gly asn lys val phe gly ala leu lys gly ala val asp gly gly leu ser ile 173

pro his ser thr lys arg phe pro gly tyr asp ser glu ser lys glu phe asn pro glu val his arg lys
CCA CAC AGT ACC AAG CGT TTC CCT GGC TAT GAC TCT GAA AGC AAA GAA TTC AAT CCT GAG GTC CAC CGC AAG 653
CCA CAC AGC ACA AAG CGA TTC CCT GGC TAT GAC TCT GAA AGC AAA GAA TTC AAT CCT GAG GTC CAC CGC AAG
pro his ser thr lys arg phe pro gly tyr asp ser glu ser lys glu phe asn pro glu val his arg lys 197

his ile phe ala gln asn val ala glu tyr met arg leu leu met glu glu asp glu asp ala tyr lys lys
CAC ATC TTT GCC CAG AAC GTT GCA GAG TAC ATG CGT CTT CTG ATG GAA GAA GAT GAA GAT GCG TAT AAA AAG 725
CAC ATC TTC GCC CAG AAT ATT GCA GAG TAC ATG CGT CTT CTG ATG GAA GAA GAT GAA GAT GCA TAT AAA AAA
his ile phe ala gln asn ile ala glu tyr met arg leu leu met glu glu asp glu asp ala tyr lys lys 221

gln phe ser gln tyr ile lys asn gly val thr ala asp gln val glu asp leu tyr lys lys ala his ala
CAG TTC TCT CAA TAC ATA AAG AAT GGT GTG ACT GCA GAC CAG GTG GAA GAC CTG TAC AAA AAG GCT CAT GCA 797
CAG TTC TCC CAG TAC ATA AAG AAT GGT GTC GCA GCA GAT CAG TTG GAA GAC ATA TAC AAA AAG GCT CAT GCA
gln phe ser gln tyr ile lys asn gly val ala ala asp gln leu glu asp ile tyr lys lys ala his ala 245

gly ile arg glu asn pro val his glu lys lys pro lys lys glu val lys lys lys arg trp asn arg ala
GGT ATT CGG GAG AAT CCA GTG CAC GAA AAG AAA CCC AAG AAG GAA GTC AAA AAG AAG AGG TGG AAC CGT GCC 869
GGT ATT CGG GAG AAT CCA GTG CAC GAA AAG AAA CCC AAG AAG GAA GTC AAA AAG AAG AGG TGG AAC CGT GCC
gly ile arg glu asn pro val his glu lys lys pro lys lys glu val lys lys lys arg trp asn arg ala 269

lys leu ser leu glu gln lys lys asp arg val ala gln lys lys ala ser phe leu arg ala gln glu lys
AAA CTG TCT CTG GAA CAG AAA AAG GAT CGT GTT GCT CAG AAA AAA GCC AGT TTC CTC AGA GCT CAG GAG AAG 941
AAA TTG TCT CTG GAA CAG AAA AAG GAC CGT GTT GCT CAG AAA AAA GCC AGT TTC CTC AGA GCT CAG GAG AAG
lys leu ser leu glu gln lys lys asp arg val ala gln lys lys ala ser phe leu arg ala gln glu lys 293

ala asp ser ***
GCA GAC AGC TAA ATGGATCATGTTGCAATTTTCTAAGTGTGTGCAGCAcggaattc                                  989
GCA GAC AGC TAA ATGGATTATGTTGCAATTTTCTAAGTGTGTGCAGCAAAAAAATcggaattc
ala asp ser ***                                                                               296
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FIG. 1. Nucleotide and derived amino acid sequences of *Xenopus* L5a and L5b cDNAs. Nucleotides are numbered relative to the first base of the L5a cDNA. The synthetic *Eco*RI linker sequences added during construction of the cDNA library are in small letters. Nucleotide and amino acid sequence differences are underlined.

regions. Primer extension analysis of tadpole poly(A)$^+$ RNA also indicated that the L5a cDNA lacks 14 nucleotides of the 5′ untranslated region (data not shown). Southern blot analysis of genomic DNA indicated the presence of two L5 genes in *Xenopus laevis* (data not shown). Comparison of the two L5 cDNA sequences reveals that these two gene copies have diverged somewhat since duplication of the *Xenopus* genome occurred some 30 million years ago (4). The majority of nucleotide differences within the L5-coding regions give rise to either silent (33 of 40) or conservative (6 of 40) substitutions. This divergence is comparable to that observed for the duplicated ribosomal protein L1 genes in *X.*

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                    10         20         30         40         50         60
Xl  L5a     MGFVKVVKNK AYFKRYQVKF RRRREGKTDY YARKRLVIQD KNKYNTPKYR MIVRVTNRDI
Xl  L5b     MGFVKVVKNK AYFKRYQVKF RRRREGKTDY YARKRLVIQD KNKYNTPKYR MIVRVTNRDI
Rat L5-6-4  MGFVKVVKNK AYFKRYQVRF RRRREGKTDY YARKRLVIQD KNKYNTPKYR MIVRVTNRDI

                    70         80         90         100        110        120
Xl  L5a     ICQIAYARIE GDMIVCAAYA HELPKYGIKV GLTNYAAAYC TGLLLARRLL NKFGLDKVYE
Xl  L5b     ICQIAYARIE GDMIVCAAYA HELPKYGVKV GLTNYAAAYC TGLLLARRLL NKFGLDKVYE
Rat L5-6-4  ICQIAYARIE GDMIVCAAYA HELPKYGVKV GLTNYAAAYC TGLLLARRLL NRFGMDKIYE

                    130        140        150        160        170        180
Xl  L5a     GQVEVTGDEY NVESIDGEPG AFTCYLDAGL TRTTTGNKVF GALKGAVDGG LSIPHSTKRF
Xl  L5b     GQVEVTGDEY NVESVDGEPG AFTCYLDAGL TRTTTGNKVF GALKGAVDGG LSIPHSTKRF
Rat L5-6-4  GQVEVMGDEY NVESIDGQPG AFTCYLDAGL ARTTTGNKVF GALKGAVDGG LSIPHSTKRF

                    190        200        210        220        230        240
Xl  L5a     PGYDSESKEF NPEVHRKHIF AQNVAEYMRL LMEEDEDAYK KQFSQYIKNG VTADQVEDLY
Xl  L5b     PGYDSESKEF NPEVHRKHIF AQNIAEYMRL LMEEDEDAYK KQFSQYIKNG VAADQLEDIY
Rat L5-6-4  PGYDSESKEF NAEVHRKHIM GQNVADYMRY LMEEDEDAYK KQFSQYIKNM VTPDM*EEMY

                    250        260        270        280        290    296
Xl  L5a     KKAHAGIREN PVHEKKPKKE VKKKRWNRAK LSLEQKKDRV AQKKASFLRA QEKA*DS
Xl  L5b     KKAHAGIREN PVHEKKPKKE VKKKRWNRAK LSLEQKKDRV AQKKASFLRA QEKA*DS
Rat L5-6-4  KKAHAAIREN PVYEKKPKRE VKKKRWNRPK MSLAQKKDRV AQKKASFLRA QERAAES
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FIG. 2. Amino acid sequence alignment of *Xenopus* and rat L5 proteins. Conservative substitutions (I = L = V; D = E; S = T; K = R) are underlined. Nonconservative substitutions are in boldface type. Gaps are indicated by an asterisk.

laevis (25). The L5a and L5b mRNAs encode two closely related L5 proteins of similar molecular weight (M_r 34,066 and 34,036, respectively) and identical net charge (+22).

Chan and colleagues previously reported a comprehensive structural comparison of rat L5 with its homologous ribosomal proteins and other 5S rRNA-binding proteins in several eucaryotes and procaryotes (10). These analyses indicate that rat L5 is homologous to the 5S rRNA-binding ribosomal proteins YL3 in yeast and HL13 in *Halobacterium cutirubrum*. However, rat L5 shares no significant sequence similarity with several other 5S rRNA-binding proteins. These include *Escherichia coli* ribosomal proteins L5, L18, and L25 and *Xenopus* TFIIIA. Comparison of the derived *Xenopus* L5a and L5b amino acid sequences with the rat L5 protein sequence deduced from pL5-6-4 shows that the primary structures of all three proteins have been exceptionally conserved (Fig. 2). The sequences of *Xenopus* L5a and L5b are 92 and 91%, respectively, identical to the rat L5 sequence. Interestingly, the positions of nonconservative amino acid subsitutions in rat and *Xenopus* L5 proteins are not randomly distributed. The majority (13 of 16) of these differences are located between residues 200 and 275. Nazar and colleagues have reported that the corresponding carboxyl-terminal region of yeast ribosomal protein YL3 binds nonspecifically to RNA (30, 45). It remains to be determined whether the sequence divergence observed in the carboxyl region of the rat and *Xenopus* L5 proteins is relevant to specific binding interactions with their respective 5S rRNAs.

Uncoupling of L5 expression from 5S rRNA synthesis during oogenesis. The accumulation of L5 mRNA was analyzed during oogenesis to compare its regulation with that of other ribosomal protein mRNAs and 5S rRNA. The steady-state levels of L5 mRNA during oogenesis were determined by Northern (RNA) blot analysis of total RNA isolated from oocytes at each stage of development. Accumulation of the 1-kilobase L5 mRNA was comparable to that observed for the 1.3-kilobase ribosomal protein L1 mRNA (Fig. 3A). Both transcripts reached maximal levels by stage II, declined somewhat in stage VI oocytes, and were deadenylated upon oocyte maturation. This profile agrees with that observed previously for other ribosomal protein mRNAs (3, 19, 36). These earlier studies also demonstrated that synthesis of ribosomal proteins, including L5, is maximal in stage III oocytes. Therefore, L5 expression is coordinated with expression of other ribosomal proteins during oogenesis. This pattern differs markedly from the expression of both 5S rRNA and its well-characterized binding protein, TFIIIA. Previous studies have shown that maximal levels of 5S rRNA and TFIIIA are attained in immature oocytes, thereby preceding synthesis of 18S and 28S rRNAs and the accumulation of ribosomal proteins (13, 32, 34). The distinct tempo-

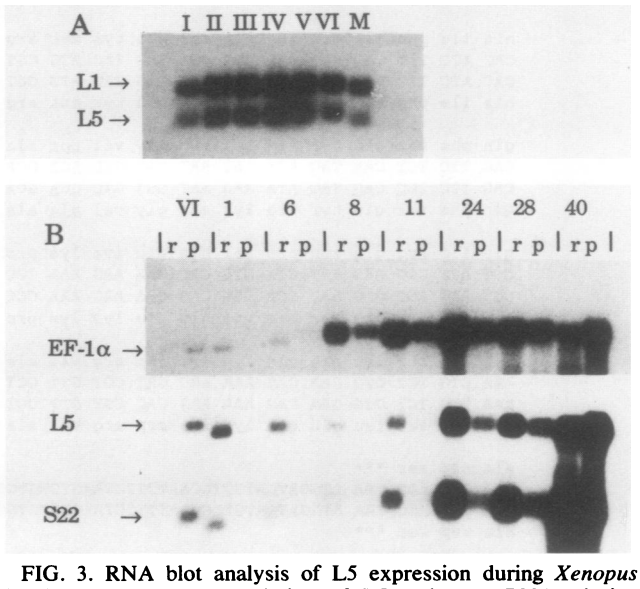

FIG. 3. RNA blot analysis of L5 expression during *Xenopus* development. (A) Accumulation of L5 and L1 mRNAs during oogenesis. Each lane contains total RNA equivalent to one oocyte. The numbers above each lane refer to the oocyte stages of Dumont (11). M refers to oocytes matured in vitro with progesterone. (B) Accumulation and polysomal association of EF-1α, L5, and S22 mRNAs during embryogenesis. Each lane contains the RNA equivalent to the nonpolysomal (r) or polysomal (p) fraction of one oocyte or embryo. Numbers above the lanes refer to the oocyte stages of Dumont (11) or embryonic stages of Nieuwkoop and Faber (31). Only the relevant regions of each autoradiogram are shown.

ral accumulation of two proteins that bind 5S rRNA in a mutually exclusive manner (5, 17, 18) necessitates a pathway in which 5S rRNA is associated initially with TFIIIA in the cytoplasm and then subsequently interacts with L5 for its nucleolar localization and 60S subunit assembly.

Translational regulation of L5 mRNA during embryogenesis. The transcriptional activation of rRNA and ribosomal protein genes commences with or shortly after the midblastula transition. Synthesis of the majority of ribosomal proteins, however, is not detected until the tailbud stage of development, when zygotic ribosomal protein mRNAs are efficiently associated with polysomes (3, 36). These studies also detected synthesis of ribosomal protein L5 soon after the midblastula stage, suggesting that this protein is subject to distinct regulation.

To determine the basis for the appearance of ribosomal protein L5 before the tailbud stage, the accumulation and translational utilization of L5 mRNA during early development was examined by Northern blot analysis of RNA extracted from polysomal and nonpolysomal fractions at different stages of development. The translational regulation of L5 mRNA was compared with the regulation of mRNAs encoding ribosomal protein S22 and EF-1α. In contrast to ribosomal protein mRNAs, which are mainly nonpolysomal before the tailbud stage, newly synthesized EF-1α mRNA is associated with polysomes at the midblastula transition (3, 21). All three mRNAs were largely polysomal in stage VI oocytes and were no longer translated in fertilized eggs (stage 1) (Fig. 3B), in agreement with previous observations (19). The levels of S22 and L5 mRNAs declined throughout cleavage stages. Both ribosomal protein mRNAs began to reaccumulate during gastrulation (stage 11) but remained predominantly nonpolysomal until the tailbud stage of development (stages 24 and 28). In contrast, the level of EF-1α mRNA increased significantly commencing at the midblastula stage (stage 8) and was accompanied by its rapid recruitment onto polysomes (Fig. 3B). The previously observed accumulation of newly synthesized L5 protein after the midblastula transition (3, 36), therefore, is not due to the efficient translation of L5 mRNA in early embryos as is observed for EF-1α mRNA. Thus, the translational utilization of L5 mRNA is coordinated with the utilization of other ribosomal protein mRNAs.

Association of newly synthesized L5 protein with 5S rRNA during embryogenesis. Previous studies have established that a large non-subunit-associated pool of L5 protein exists in HeLa cells (23, 33), in contrast to the exceptional instability of most free ribosomal proteins (26). Steitz and colleagues have recently demonstrated that L5 associates with 5S rRNA before incorporation of the L5-5S rRNA complex into the 60S subunit of mammalian cells (40). These studies revealed that as much as 50% of the non-subunit-associated 5S rRNA is bound to L5. The onset of 5S rRNA synthesis at the midblastula stage (7, 44) could serve to stably sequester newly synthesized L5 protein in the absence of ribosome assembly.

To address this possibility, extracts were prepared from embryos microinjected with either [^{35}S]methionine or [α-^{32}P]UTP and incubated with human anti-5SRNP serum. Steitz et al. (40) have shown that this antiserum recognizes the L5-5S rRNA complex in both HeLa and mouse cells but does not immunoprecipitate either intact ribosomes or free 5S rRNA. A 34-kilodalton (kDa) protein was immunoprecipitated with the anti-5SRNP serum from extracts derived from embryos ^{35}S-labeled after the midblastula transition (Fig. 4A, lane 2) and during gastrulation (Fig. 4A, lane 3). The immunoprecipitated 34-kDa protein comigrated with L5 synthesized by in vitro translation of an SP6-L5 mRNA (Fig. 4A, lane 4), and its identity was confirmed by partial proteolysis with V8 protease (data not shown). L5 protein was not present in a gastrula-stage extract immunoprecipitated with normal human serum (Fig. 4A, lane 1). The presence of other ^{35}S-labeled proteins in addition to L5 in anti-5SRNP immunoprecipitates was also observed in HeLa cell extracts by Steitz et al. (40). The basis for the immunoreactivity of these proteins with anti-5SRNP serum has not been addressed.

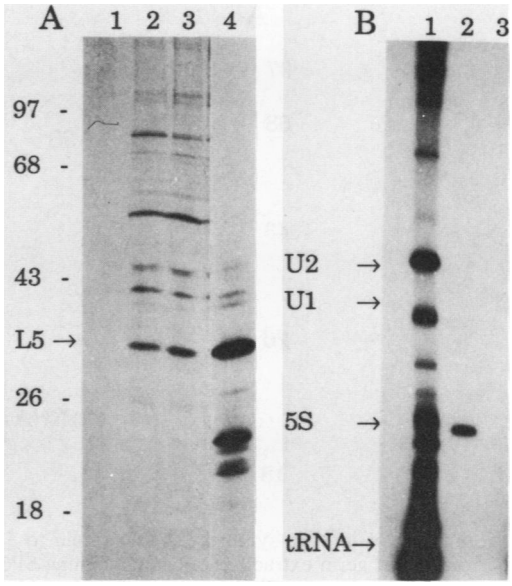

FIG. 4. Association of newly synthesized L5 protein and 5S rRNA in *Xenopus* embryos. (A) 15% SDS–polyacrylamide gel analysis of embryonic ^{35}S-labeled proteins immunoprecipitated with normal human serum (lane 1) or human anti-5SRNP serum (lanes 2 and 3). Proteins were metabolically labeled by microinjection of [^{35}S]methionine into the blastocoel of stage 8 (lanes 1 and 2) and stage 11 (lane 3) embryos, which were allowed to develop for an additional 6 and 12 h, respectively. Each lane contains the protein immunoprecipitated from two embryos. Lane 4 contains ^{35}S-labeled L5 protein synthesized by in vitro translation of SP6-L5 mRNA in wheat germ extract. Numbers on the left indicate molecular sizes in kilodaltons. (B) 8% polyacrylamide–8 M urea gel analysis of embryonic ^{32}P-labeled RNAs immunoprecipitated with human anti-5SRNP serum (lane 2) or normal human serum (lane 3). RNAs were metabolically labeled by microinjection of [α-^{32}P]UTP into fertilized eggs, which were allowed to develop for 12 h. Lane 1 contains total RNA. Each lane contains the total or immunoprecipitated RNA equivalent to two embryos.

Newly synthesized 5S rRNA was also immunoprecipitated from a ^{32}P-labeled gastrula-stage extract with anti-5SRNP serum (Fig. 4B, lane 2) but did not react with normal human serum (Fig. 4B, lane 3). Since it was not determined whether saturating amounts of antiserum were present in these immunoprecipitations, these assays do not provide a quantitative measurement of the fraction of newly synthesized L5 or 5S rRNA present in the complex. These results do not rule out either the exchange of newly synthesized L5 into a preexisting maternal L5-5S rRNA pool or the formation of a complex between newly synthesized 5S rRNA and a preexisting maternal L5 pool. These results suggest strongly, however, that both newly synthesized L5 protein and 5S rRNA accumulate in concert as a stable complex in

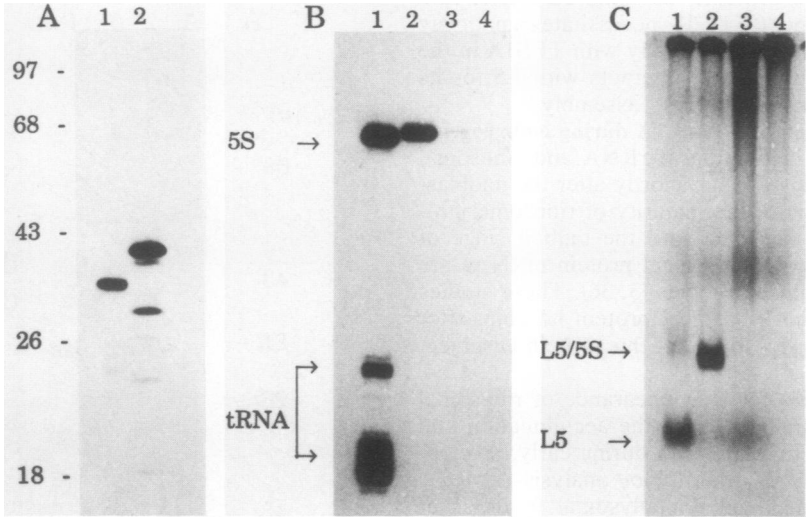

FIG. 5. Binding of in vitro-synthesized L5 protein to 5S rRNA. (A) 15% SDS–polyacrylamide gel analysis of ^{35}S-labeled translation products from wheat germ extract reactions containing SP6-L5 mRNA (lane 1) and SP6-L1 mRNA (lane 2). Numbers on the left indicate molecular sizes in kilodaltons. (B) 8% polyacrylamide–8 M urea gel analysis of ^{32}P-labeled immature oocyte RNAs incubated with L5 (lanes 2 and 3) and L1 (lane 4) in vitro translation reaction mixtures and immunoprecipitated with human anti-5SRNP serum. Lane 1 contains total immature oocyte RNA. Lane 3 is the same as lane 2 except that the binding reaction was performed in the presence of 5 μg of gel-purified *Xenopus* 5S rRNA. (C) Gel retardation analysis of L5-5S rRNA complex formation. Postribosomal supernatants containing ^{35}S-labeled L5 (lanes 1 and 2) and L1 (lanes 3 and 4) wheat germ extract translation products were incubated in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of gel-purified 5S rRNA and analyzed by 8% native polyacrylamide gel electrophoresis.

embryonic stages preceding the assembly of ribosomal subunits.

Binding of 5S RNA by in vitro-synthesized L5 protein. The stable and specific complex formed between L5 and 5S rRNA in the absence of ribosome assembly provides a well-defined system for analysis of structural determinants of RNA-protein recognition. Huber and Wool (17) have identified the L5-binding site on rat 5S rRNA by use of α-sarcin protection assays. In contrast, little is known about the L5 sequences that are required for 5S rRNA-binding activity. Specific 5S rRNA binding of either purified yeast YL3 or mammalian L5 has not been reported to date. The inability to reconstitute the L5-5S rRNA complex from purified components has been attributed at least in part to the poor solubility of L5 protein extracted from subunits under denaturing conditions (17, 30, 45).

As an alternative approach to examining this aspect of L5 function, a synthetic mRNA encoding *Xenopus* L5b was translated in vitro in wheat germ extract, and the resulting protein product was analyzed for specific 5S rRNA-binding activity. The SP6-L5b mRNA was efficiently translated into a 34-kDa protein (Fig. 5A, lane 1). This in vitro translation product comigrated with L5 protein extracted from 60S subunits, and identical proteolytic fragments were obtained for both proteins upon partial digestion with V8 protease (data not shown). Wheat germ translation reaction mixtures containing either SP6-L5 mRNA or SP6-L1 mRNA (Fig. 5A, lane 2) were incubated with ^{32}P-labeled immature oocyte RNA in the presence of excess unlabeled tRNA or 5S rRNA competitors. The reaction mixtures were then incubated with human anti-5SRNP serum, and the immunoprecipitated RNAs were analyzed by denaturing polyacrylamide gel electrophoresis. 5S rRNA was specifically immunoprecipitated from L5 in an vitro translation reaction (Fig. 5B, lane 2). The immunoprecipitation of labeled 5S rRNA was significantly reduced by the addition of unlabeled 5S rRNA competitor (Fig. 5B, lane 3), demonstrating the specificity of 5S rRNA binding. The precipitation of labeled 5S rRNA was not due to its association with endogenous wheat germ L5 protein. No detectable 5S rRNA was precipitated with anti-5SRNP serum in translation reactions containing SP6-L1 mRNA (Fig. 5B, lane 4). 5S rRNA was not precipitated from L5 reactions with normal human serum (data not shown). These results indicate L5 protein synthesized in vitro and purified 5S rRNA form a complex identical in antigenic properties to the L5-5S rRNA particle present in mammalian cells.

The 5S rRNA-binding activity of in vitro-synthesized L5 protein was also assayed by electrophoresis of the L5-5S rRNA complex on nondenaturing gels. In these experiments, specific RNA binding was detected by the altered electrophoretic mobility of ^{35}S-labeled L5 protein in the presence of 5S rRNA. ^{35}S-labeled L5 protein migrated as a discrete band in the absence of 5S rRNA (Fig. 5C, lane 1). The addition of purified 5S rRNA generated a new band with a reduced electrophoretic mobility and a concurrent loss of the faster-migrating species (Fig. 5C, lane 2). Neither this slower-migrating complex nor any other altered mobilities were observed when 5S rRNA was added to binding reactions containing ^{35}S-labeled L1 protein (Fig. 5C, lanes 3, 4). These results further confirm the ability of in vitro-synthesized L5 to bind specifically to 5S rRNA and provide a basis for delineating the RNA-binding domain of this ribosomal protein.

DISCUSSION

Developmental regulation of *Xenopus* L5 gene expression. The results presented above indicate that the expression of ribosomal protein L5 can either coincide with 5S rRNA synthesis and ribosome assembly or be controlled independently of these events at different stages of *Xenopus* development. During oogenesis, the abundance and translational activity of L5 mRNA is coordinately regulated with other

ribosomal protein mRNAs. The maximal synthesis of ribosomal proteins coincides with transcription from amplified nucleoli and ribosome assembly (3, 9). These events are preceded by the accumulation of the 5S rRNA-TFIIIA storage particle in the cytoplasm of immature oocytes (13, 32, 34). This substantial lag between maximal 5S rRNA transcription and the appearance of L5 differs markedly from the situation in other systems, where both of these ribosomal constituents accumulate simultaneously.

In mammalian cells, newly synthesized 5S rRNA rapidly associates with L5 to form a complex that is a discrete precursor to 60S subunit assembly (40). 5S rRNA synthesized in vitro in isolated nuclei from mammalian cells (40) or in yeast nuclear extracts (5) is bound to L5, providing evidence that a nuclear pool of L5 is available to sequester 5S rRNA for its nucleolar localization in these cells. The cytoplasmic TFIIIA-5S rRNA storage particle present in immature *Xenopus* oocytes must therefore be dissociated in order to form the L5-5S rRNA complex that can participate in 60S subunit assembly. Since the TFIIIA-5S rRNA complex is exceedingly stable and since both TFIIIA and L5 recognize a mutually exclusive, common binding site on 5S rRNA (5, 17, 18), it will be of interest to determine the mechanism for this exchange of 5S rRNA-binding proteins.

During embryogenesis, L5 mRNA is subject to the same translational regulation that encompasses other ribosomal protein mRNAs. These transcripts begin to accumulate after the midblastula stage but remain predominantly nonpolysomal before the tailbud stage of development (3, 36). The low level of polysomal L5 mRNA before this stage, however, is sufficient to direct the accumulation of L5 protein, which stably associates with 5S rRNA before new ribosomes are assembled. In contrast, the inefficient translation of other ribosomal protein mRNAs in early embryos does not generate a comparable accumulation of these proteins because of their rapid degradation in the absence of subunit assembly (35). Thus, although translational control is a major level for the coordinate regulation of ribosomal protein mRNAs during early embryogenesis, it does not generate identical patterns of accumulation for these proteins before ribosome assembly. The mechanism by which ribosomal protein mRNAs are selectively excluded from polysomes during early embryogenesis remains to be determined. The expression of systematically altered L5 genes in microinjected eggs will facilitate the identification of *cis*-acting elements required for this translational control.

These analyses of L5 expression, in addition to previous studies (reviewed in reference 43), reinforce the conclusion that the synthesis and accumulation of rRNAs and individual ribosomal proteins are independently regulated events that precede the assembly of new ribosomes in the swimming tadpole. Recent studies reveal that this apparent paradox includes other components essential to the translational apparatus or ribosome assembly. The efficient synthesis of EF-1α that commences at the midblastula stage (21) does not correlate with a significant increase in overall protein synthesis (41). The accumulation of nucleolin, a nucleolar protein implicated in the initial steps of ribosome biogenesis, likewise precedes the translation of ribosomal protein mRNAs and ribosome assembly (8). Thus, the temporal expression of all genes encoding translational components characterized to date is not synchronized with the requirement for ribosome assembly during *Xenopus* development.

5S rRNA-binding activity of in vitro-synthesized L5. Ribosomal protein L5 binds specifically to 5S rRNA in vivo and has been implicated in its nucleolar localization (10, 40). Whereas the L5-binding site on 5S rRNA has been identified (17), the L5 sequences comprising the 5S-rRNA binding domain remain uncharacterized. The results described in this report demonstrate that L5 protein synthesized in vitro binds specifically to 5S rRNA. The ability to use the native L5 translation product for these binding studies circumvents the poor solubility and nonspecific RNA-binding activity of L5 protein purified from 60S subunits or released from the L5-5S rRNA complex under denaturing conditions (17, 30, 45). Recent analyses have revealed the presence of a conserved octapeptide, designated the ribonucleoprotein consensus sequence, in a number of eucaryotic RNA-binding proteins (reviewed in reference 1). Query et al. (37) have demonstrated that this conserved sequence comprises part of the U1 small nuclear RNA-binding domain of the 70,000-molecular-weight U1 small nuclear ribonucleoprotein protein. The absence of this highly conserved motif from L5 indicates that alternative structures contribute to its specific interaction with 5S rRNA. It is particularly interesting that although L5 and TFIIIA recognize a common binding site on 5S rRNA, these two proteins share no sequence similarity and are subject to distinct developmental regulation. Likewise, it remains to be determined whether the two *Xenopus* L5 proteins have different affinities for oocyte and somatic-type 5S rRNAs. Delineation of the 5S rRNA-binding domain and other functional regions within L5 will address these problems as well as the mechanism by which 5S rRNA is exchanged from a cytoplasmic TFIIIA-5S rRNA storage particle into the L5-5S rRNA complex that becomes localized within the nucleolus and is assembled into 60S subunits.

ACKNOWLEDGMENTS

I thank Ira Wool and Doug Melton for generously providing pL5-6-4 and the embryonic cDNA library, respectively. I am grateful to Joan Steitz for generously providing anti-5SRNP sera and protocols for immunoprecipitation. I thank Paul Huber and John Woolford for many useful discussions. Brett Keiper, Ranjan Sen, and Susan Varnum improved the manuscript with helpful comments.

This work was supported by Public Health Service grant HD-17691 from the National Institutes of Health and basic research grant 1-1040 from the March of Dimes Birth Defects Foundation.

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