# Small Nuclear RNA Transcription and Ribonucleoprotein Assembly in Early *Xenopus* Development

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ABSTRACT The *Xenopus* egg and embryo, throughout the transcriptionally inactive early cleavage period, were found to contain a store of approximately $8 \times 10^8$ molecules of the small nuclear RNA (snRNA) U1, sufficient for 4,000–8,000 nuclei. In addition, when transcription is activated at the twelfth cleavage (4,000 cell-stage), the snRNAs U1, U2, U4, U5, and U6 are major RNA polymerase II products. From the twelfth cleavage to gastrulation, U1 RNA increases sevenfold in 4 h, paralleling a similar increase in nuclear number. This level of snRNA transcription is much greater than that typical of somatic cells, implying a higher rate of U1 transcription or a greater number of U1 genes active in the embryo. The *Xenopus* egg also contains snRNP proteins, since it has the capacity to package exogenously added snRNA into immunoprecipitable snRNP particles, which resemble endogenous particles in both sedimentation coefficient and T1 RNase digestibility. SnRNP proteins may recognize conserved secondary structure of U1 snRNA since efficient packaging of both mouse and *Drosophila* U1 RNAs, differing 30% in sequence, occurs. The *Xenopus* egg and embryo can be used to pose a number of interesting questions about the transcription, assembly, and function of snRNA.

Small nuclear RNA molecules (snRNA) are an abundant component of most eucaryotic cells (11, 19, 38, 48, 59). Mammalian nuclei contain approximately $10^6$ molecules of the most abundant small nuclear RNA, U1, per nucleus, while the nuclei of the cellular slime mold, *Dictyostelium*, contain fewer ($3 \times 10^3$ per nucleus) but significant numbers of U1-like molecules (44, 53, 54). Six small nuclear RNA species (U1-U6), ranging in size from 90 to 216 nucleotides, were originally identified in mammalian nuclei and found to have several features in common: all (except U6) possess $m^{2,2,7}G$ caps at the 5′ end, all are rich in uridine residues, and all are primarily nuclear molecules (31, 48, 59; for a review see reference 8). In addition, all except U3 are immunoprecipitable from cell extracts by antisera from human systemic lupus erythematosus patients (25, 27). Lupus antisera recognize two separate antigens associated with small nuclear RNAs (25). Both of these antigens have been shown to reside on RNA-protein particles with sedimentation coefficients of approximately 11S (8, 26, 32, 35). One of the antigenic determinants, designated RNP, is associated with a ribonucleoprotein particle containing a molecule of U1 snRNA and approximately seven different proteins ranging in size from 10,000 to 68,000 daltons (8, 17, 25, 51). The other antigen, Sm, appears to be a protein(s) present on a number of ribonucleoprotein particles, each of which contains one molecule of small nuclear RNA (snRNA), either U1, U2, U4, U5, or U6, and most but not all of the proteins found in the RNP particle (26, 51). Certain common structural features have been found in each of the RNAs, U1, U2, U4, U5, and U6, despite their difference in size; this may provide an explanation for the binding by each RNA to the same set of core snRNP proteins (22, 36).

Several lines of evidence support the involvement of snRNA in the processing of messenger RNA precursors. (*a*) The 5′ end of U1 RNA shows striking complementarity to a "consensus" sequence derived from compiling sequence data for many of the exon-intron junctions present in mRNA precursors (26, 33, 39). (*b*) When purified U1 RNA is incubated (under hybridization conditions) with a 16-nucleotide DNA sequence that mimics the consensus sequence, a short sequence at the 5′ end of U1 is found to hybridize (24). (*c*) In vivo, a large proportion of snRNPs can be found tightly bound to heterogeneous nuclear RNA-protein particles (mRNA precursors) (see reference 60 for a review; 9, 10, 34). (*d*) Lastly, when anti-RNP and anti-Sm antisera are added to adenovirus-infected nuclei in which viral mRNA splicing



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normally occurs, the antisera reduce or completely prevent correct splicing of viral precursor RNA molecules (57).

The cells of the early *Xenopus* embryo undergo a number of rapid and synchronous developmental changes. The complex division and transcriptional patterns of somatic cells are gradually established. Thus this system offers unique opportunities for examining questions of transcription, mRNA precursor processing, and the relation of RNA processing to development. We have examined snRNAs, thought to be involved in mRNA precursor processing, in the developmental context of the *Xenopus* embryo. Following fertilization, rapid cleavage ensues and all nuclei present up to the twelfth cleavage are transcriptionally inactive (28). At the twelfth cleavage, the embryo consists of 4,000 cells and has reached the midblastula stage of development. Transcription of a subset of the genome is abruptly activated (3, 4, 28, 29, 55). Also, at this time, cell division becomes asynchronous and cell motility is first observed (28). At these early stages, the RNA present in the *Xenopus* egg and embryo differs from that present in later stages, the egg containing a large store of maternal mRNA which persists through the midblastula stage. The sequence complexity of maternal RNA (and thus pre-midblastula RNA) is much higher than that found in normal somatic cells, and this complexity has been seen to decrease by 30% during the blastula-gastrula period (12). In addition, the maternal RNA of mature *Xenopus* oocytes has been reported more recently to have a complexity intermediate between somatic cell messenger RNA (fully processed) and nuclear RNA (unprocessed mRNA precursors) (1, 45).

Because of the different nuclear and transcriptional states present in the early *Xenopus* embryo and the possible requirement for processing of stored maternal RNA for normal development, we undertook a study to determine when small nuclear RNA molecules and their associated proteins are made and how they function in the *Xenopus* embryo. We report here that: (*a*) SnRNAs are among the major RNA products made at the onset of embryonic transcription. (*b*) During the period from the midblastula stage until early gastrula stage, snRNAs are transcribed at a rate many times the rate seen in somatic cells. (*c*) Despite a lack of any early transcription, the snRNA U1 is stored in the transcriptionally inactive early embryo in sufficient amounts for 4,000–8,000 nuclei. (*d*) SnRNP proteins must also be present in the unfertilized egg, since we find that radioactive U1 and U2 injected into the egg are assembled into immunoprecipitable entities. (*e*) In addition, as also reported by De Robertis (16), we find that the *Xenopus* snRNP protein(s) have the capacity to assemble onto injected snRNA from distantly related species. Specifically, we find that *Xenopus* snRNP protein(s) can assemble onto both *Drosophila* and mouse U1 snRNAs, which differ greatly in their primary sequence, suggesting that snRNP protein(s) recognize conserved secondary structures rather than sequences. (*f*) Using two types of physical characterization, we show that *Xenopus* U1 at least, when injected into fertilized eggs, is assembled into a RNA-protein structure resembling native snRNP particles.

## MATERIALS AND METHODS

*Materials:* $\alpha$-$^{32}$P-rUTP (410 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL); $^{32}$P-$PO_4$- and $\alpha$-$^{32}$P-dCTP from New England Nuclear (Boston, MA). Acrylamide, urea, alkaline phosphatase, Sigma 104 Phosphatase Substrate, and ONPG were obtained from Sigma Chemical Co. (St. Louis, MO), T1 RNAse from Calbiochem-Behring Corp. (San Diego, CA). *E. coli* $\beta$-galactosidase and Staph A prepared by the method of Kessler (21) were the gifts of Dr. Brian Craine and Dr. Gary Firestone. The *Xenopus* A6 cells were obtained from the American Type Culture Collection (Rockville, MD), the *Drosophila* Kc cells from D. Hogness, and the mouse S49 cells from P. Coffino. The human U1 clone, pU1.15, was generously provided by Dr. Alan Weiner (14) and the *Xenopus* ribosomal 28S rRNA clone (gene 315) by Dr. Ronald Reeder. Anti-RNP (Ag) and anti-Sm,RNP (Am) antisera from systemic lupus erythematosus patients were the generous gift of Dr. Joan Steitz. (The antisera were put through two 30% ammonium sulfate precipitations, dialyzed versus 20 mM potassium phosphate, pH 6.3, and used at a concentration of 10 mg/ml [26]). *Xenopus laevis* frogs were obtained from South African Snake Farm (Cape Providence, South Africa) and Charles Sullivan (Nashville, TN).

*Labeling of Embryonic and Tissue Culture Cell RNA:* Labeled embryonic RNA was obtained by injecting 500 nl of $\alpha$-$^{32}$P-rUTP (1–2 $\mu$Ci/egg; in 10 mM potassium phosphate 0.1 mM EDTA) into the animal pole of fertilized, dejellied eggs placed in 5% Ficoll (type 400; Sigma Chemical Co.) and MMR (100 mM NaCl, 2 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 5 mM HEPES, 0.1 mM EDTA, pH 7.8) (28). The embryos were allowed to develop for 10 to 12 h (several hours beyond the onset of transcription) in MMR/4 and lysed in 0.2–0.8 ml of 100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$. The yolk was removed by spinning for 3 min at room temperature in an Eppendorf centrifuge. For preparative isolation of specific radioactive RNA species, the RNA was extracted from the lysate with an equal volume of phenol:chloroform (1:1; two to four times), followed by chloroform extraction (two times) and ethanol precipitation. The RNA was then fractionated on acrylamide-urea gels as described below.

Labeled *Drosophila*, mouse, and *Xenopus* tissue culture cell RNAs were extracted from Kc, S49, and A6 cell lines, respectively. Approximately $10^8$ cells, labeled overnight with several mCi of $^{32}PO_4^-$, were lysed in 5 M guanidine thiocyanate, 50 mM Tris, pH 7.67, 10 mM EDTA, 5% $\beta$-meraptoethanol (BME). This was extracted with phenol:$CHCl_3$, $CHCl_3$, EtOH-precipitated, and fractionated preparatively as described for embryonic RNA.

*Gel Electrophoresis of RNA:* Unless stated otherwise, all gels contained 5% acrylamide and 7 M urea. The electrophoresis buffer contained 100 mM Tris-HCl, 100 mM boric acid, and 2 mM EDTA (pH 8.25). RNA samples were resuspended in formamide loading buffer with Bromophenol Blue and xylene cyanol as dye markers. Gels were electrophoresed at 1,500 V for 2–3 h (unless otherwise stated) and immediately exposed at −70°C using X-Omat AR-5 film and a Kodak Quanta III intensifying screen. Densitometry of autoradiographs was performed with a Zeineh densitometer, and was shown by standard curves to linearly measure relative levels of radioactive RNA. Specific RNA species were extracted from the gel by excision of the piece of acrylamide containing the desired band and elution for several hours at 4°C in 0.4–0.8 ml 100 mM NaCl, 20 mM Tris-HCl, pH 7.5, 20 mM $MgCl_2$. The RNA eluate was ethanol-precipitated in siliconized tubes without the addition of carrier RNA.

*Immunoprecipitations:* Labeled embryos or embryos injected (near the animal pole) with radioactive snRNA were lysed at 10–12 h or 3 h, respectively, in 100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$ (50–100 $\mu$l per embryo). The yolk was removed by a 3-min spin in an Eppendorf centrifuge. Antiserum (2–5 $\mu$l) was added at 0°C for 10–30 min, followed by addition of 10% Staph A cross-linked bacterial coats (10 $\mu$l). After a further 10–30 min on ice, the immunoprecipitate and Staph A were recovered by a 3-min spin in the Eppendorf centrifuge. The supernatant was immediately extracted with an equal volume of phenol:$CHCl_3$ (1:1). The pellet was washed three to five times with 0.4 ml of 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 0.05% Nonidet P-40 (NP-40) and extracted with phenol:$CHCl_3$. Both pellet and supernatant were extracted further with phenol:$CHCl_3$ (1:1, two to four times) and $CHCl_3$ (two times), prior to EtOH precipitation. Carrier RNA (20 $\mu$g yeast tRNA) was added to the immunoprecipitates to facilitate ethanol precipitation.

*T1 RNAse Digestion of snRNP and snRNA:* To analyze the U1 fragments produced by T1 RNAse digestion of in vivo labeled U1 snRNP particles, ten fertilized *Xenopus* eggs were injected with $\alpha$-$^{32}$P-rUTP, allowed to develop for 10 h, and lysed in 400 $\mu$l of 100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$. After removal of the yolk, the embryo extract was split into two parts. 30 $\mu$g of T1 RNAse was added to one part. Both parts were incubated at 0°C for 30 min, at which time 2 $\mu$l of anti-RNP antiserum was added. Immunoprecipitation was performed and the RNA present in both precipitate and supernatant was analyzed as described.

To analyze the U1 fragments produced by T1 RNAse digestion of injected *Xenopus* U1, ~350 cpm of in vivo labeled *Xenopus* U1 were injected into 10 eggs, allowed to incubate for several hours, and treated exactly as above, one half being digested with T1 RNAse prior to immunoprecipitation with 5 $\mu$l of anti-RNP antiserum.

The T1 fragments produced by digestion of isolated U1 RNA were analyzed

in reaction mixtures containing 400 μl of 100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 2 μl yeast tRNA (20 μg), and 10 μl of labeled *Xenopus* embryonic U1 RNA in $H_2O$, incubated for 5 min on ice. 5 μl of varying concentrations of T1 RNAse in 10 mM Tris-HCl, 2 mM EDTA were then added and incubation was continued for 5 min on ice. The RNA was phenol:$CHCl_3$ extracted, precipitated, and separated on a 5% acrylamide-urea gel.

*Hybridization Analysis of Stored U1 RNA:* A large number of embryos were fertilized at $t = 0$ and staged by carefully following the early cleavages. 75 embryos were withdrawn at different developmental stages, and the nucleic acid was extracted by solubilization in guanidine thiocyanate as described above for tissue culture cell RNA. The purified RNA (and DNA) of approximately 3.75 embryos was dissolved in $H_2O$, mixed with one-fifth volume of 20% sucrose, 1% sarkosyl, 0.05% Bromophenol Blue, 100 mM EDTA, and electrophoresed on a 10% acrylamide gel (no urea) at 200 V. The electrophoresis buffer was 80 mM Tris-HCl, 78 mM boric acid, 1 mM EDTA, pH 8.3. After staining with ethidium bromide, the RNA was transferred electrophoretically to DBM paper with 50 mM sodium acetate, pH 5.8, 1 mM EDTA as transfer buffer (43). The blot was hybridized with a T4 DNA polymerase-labeled 196 base pair fragment that contained an entire human U1 gene ($10^6$ cpm) in 50% formamide, five times SSC, 250 μg/ml yeast RNA, 1% glycine, 0.5% NP-40, five times Denhardt's mix, 50 mM sodium phosphate, pH 7, at 42°C. The blot was washed with two times SSC at room temperature for 2 h, exposed to X-Omat AR-5 film for 20 h with an intensifying screen, and the resulting autoradiograph was scanned with a Zeineh densitometer. To measure the amount of ribosomal RNA in each sample, the same blot was hybridized to a T4 DNA polymerase-labeled 28S RNA probe (clone 315) and processed in the same manner described above.

*Determination of S Values:* Labeled *Xenopus* U1 RNA was injected into 20 eggs. After 4 h the eggs were lysed in 0.4 ml of 100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, and the yolk was removed as described above. Labeled *Xenopus* tRNA and 5S RNA, alkaline phosphatase (20 μg), and β-galactosidase (2 U) were added as size markers in 50 μl of $H_2O$ to the extract. The mixture was added to a 12 ml 15–30% sucrose gradient (100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1.5 mM $MgCl_2$) and centrifuged in an SW40 rotor (Beckman Instruments, Inc., Palo Alto, CA) at 35,000 rpm for 22 h at 4°C. Fractions (0.3 ml) were collected, mixed with 5 M guanidine thiocyanate, 50 mM Tris-HCl, pH 7.6, 10 mM EDTA, 5% BME, and the RNA was extracted with phenol:$CHCl_3$ (1:1), followed by EtOH precipitation. The RNA in each fraction was visualized by separation on an acrylamide-urea gel. An aliquot of the fractions was analyzed to determine the presence of β-galactosidase and alkaline phosphatase (prior to RNA extraction). The mobility of the standards (fraction number) was plotted relative to their known S value, and the S value of U1 (after injection) was determined from this plot. A parallel gradient was run with markers, 40 μg of yeast tRNA, and isolated radioactive U1, to determine the S value of the latter.

## RESULTS

### *SnRNAs Are Major Transcripts in the Early Xenopus Embryo*

When fertilized eggs were injected with $^{32}$P-rUTP at the one-cell stage and allowed to develop for various lengths of time before extraction of nucleic acids and fractionation on a polyacrylamide gel, the pattern of transcription shown in Fig. 1 was revealed, as previously described (28). A limited number of discrete transcripts are apparent; the majority of new transcripts are of low molecular weight. (In Fig. 1, a large amount of high molecular weight material is seen. This material is synthesized even in lysed eggs when $^{32}$P-rUTP is added and is sensitive to DNAse. In other injections, particularly those in Fig. 2, little or no high molecular weight material is observed, while high levels of the low molecular weight transcripts are observed.) The transcripts seen are tRNAs and molecules of a size evocative of the small nuclear RNA molecules, U1, U2, etc. Consistent with the earlier studies of others (2, 4, 5, 28), the embryos appear transcriptionally inactive at early cleavage stages and transcription becomes active at 6–7 h after fertilization. Injection of [$^3$H]uridine into fertilized eggs and autoradiography of sectioned embryos at various times has confirmed that transcription is not seen in individual nuclei until 6–7 h after fertilization (28).

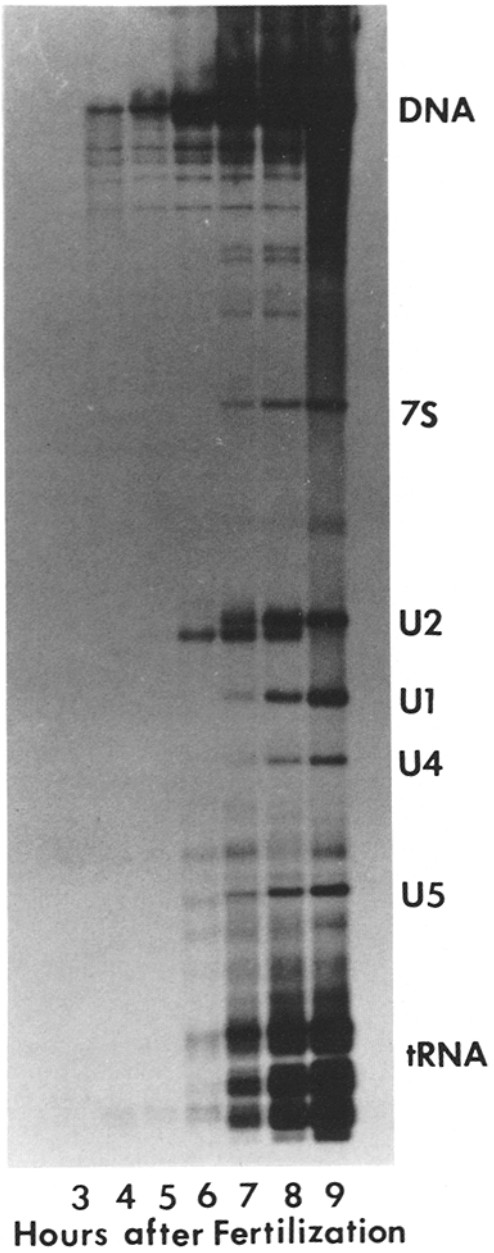


FIGURE 1 SnRNA transcription in early *Xenopus* embryos. Fertilized eggs were injected at the one-cell stage with 0.5 μCi of α-$^{32}$P-rUTP in 25 nl of 10 mM potassium phosphate, 0.1 mM EDTA, and allowed to develop. At different times following fertilization, the labeled embryos were lysed in 100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1% SDS, and the RNA was extracted and fractionated as described in Materials and Methods. Approximately two embryos were used per time point, normalizing for total counts per minute injected. An autoradiograph of the gel is shown. RNA from embryos allowed to develop for 3, 4, 5, 6, 7, 8, and 9 h following fertilization was fractionated as shown. The high molecular weight bands labeled "DNA" are synthesized even if the α-$^{32}$P-rUTP is added to previously lysed eggs.

Active transcription of snRNAs at this time in development was confirmed by immunoprecipitation (Fig. 2). One-cell embryos were injected with labeled rUTP and the embryos were allowed to develop several hours past the onset of transcription. The embryos were then lysed in buffer, and

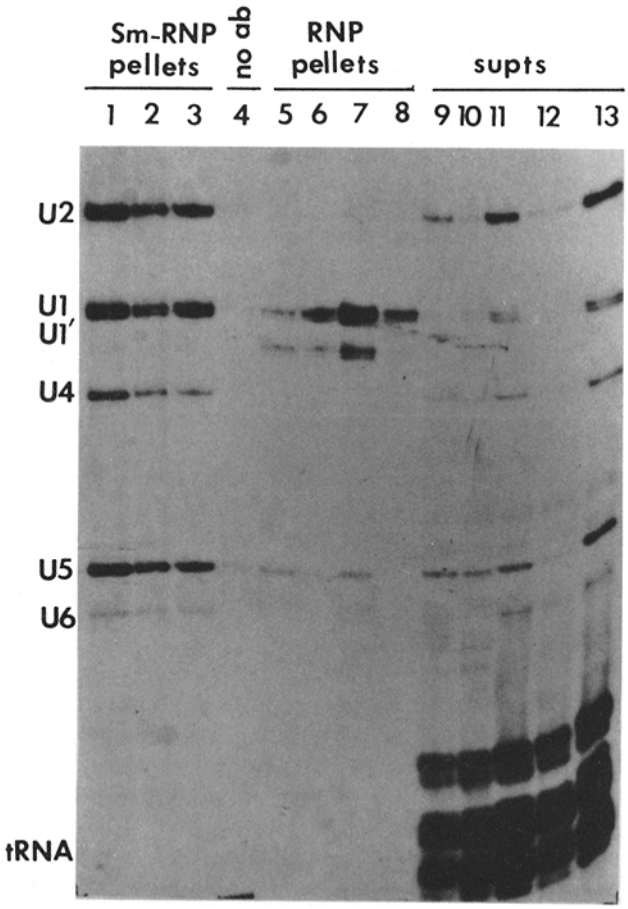


FIGURE 2 Identification of many early *Xenopus* transcripts as snRNAs. *Xenopus* embryos injected at the one-cell stage with 1–2 μCi α-$^{32}$P-rUTP were allowed to develop several hours past the onset of transcription. For each lane shown, two such labeled eggs were used. Antiserum was added to the embryo extracts and immunoprecipitation performed as described in Materials and Methods. The radioactive RNAs present in the immunoprecipitates and supernatants were separated on a 5% acrylamide-urea gel and autoradiographed for 76 h. The RNA immunoprecipitated by anti-Sm-RNP antiserum is shown in lanes *1–3* and by anti-RNP antiserum in lanes *5–8*. (Five times the normal amount of Staph A bacteria was added to the extract in the lane 6.) As a control, the extract of two labeled eggs was carried through the immunoprecipitation procedure but without the addition of antiserum (lane *4*). The radioactive RNA present in the supernatants of the anti-RNP immunoprecipitations is shown in lanes *9* and *10* and of anti-Sm-RNP immunoprecipitations in lanes *11* and *12* while lane *13* contains that supernatant of the control immunoprecipitation without antiserum). The RNAs are designated *U1, U2, tRNA*, etc., from the results of these immunoprecipitation and from comparison with radioactive size markers (markers not shown). The upper portion of the autoradiograph was essentially empty except for labeled 7S RNA in the supernatant lanes and a slight smear of DNA in some lanes.

antiserum against RNP or Sm-RNP from human lupus patients was added. (The Sm-RNP antiserum reacts with both snRNP antigens, RNP and Sm.) Nucleic acid was extracted from the immunoprecipitate and was resolved by gel fractionation (Fig. 2). Anti-Sm-RNP antiserum (Fig. 2, lanes 1–3) precipitates entities containing RNAs the size of U2, U1, U4, U5, and U6 (size markers not shown on this gel). The pattern seen is almost identical to that of immunoprecipitated human snRNA (16, 26). In this and other experiments, *Xenopus* early embryonic U1 runs as a doublet. In the mouse, two species of U1 are also seen, different in sequence but not in length, while, in humans and chickens, U1 runs as a single species (18, 25, 26, 40). Anti-RNP antiserum (Fig. 2, lanes 5–8) immunoprecipitates entities containing the U1 doublet and sometimes a doublet (U1′) of lower molecular weight, probably corresponding to specific U1 degradation fragments created by cleavage of both U1 species at a common RNAse-sensitive site. (The U1′ doublet appears to be missing sequences at the 5′ ends of the molecules, since embryonic U1 labeled at the 3′-end with $^{32}$P-pCp gives U1′-sized degradation products when injected into *Xenopus* eggs and immunoprecipitated with anti-RNP [data not shown; see Results below for experimental protocol]). *Xenopus* U1′ species may be similar to mouse U1a*. The immunoprecipitation of a U5-sized RNA by anti-RNP (Fig. 2, lanes *5–8*) probably represents nonspecific binding of this RNA to the Staph A immunoadsorbant, since this band is also relatively enriched even when no antibody is present (Fig. 2, lane *4*). In summary, a large fraction of the small RNA species labeled early after the onset of transcription in *Xenopus* embryos consists of small nuclear RNAs. The only other labeled embryonic RNA transcripts present in abundance at this early time include tRNAs and the 7S cytoplasmic RNA (49; assignment of this RNA, which is identical in size to SRP 7S RNA, as *Xenopus* 7S is based solely on mobility).

Early transcripts in *Xenopus* are unusually enriched in snRNAs as compared to the transcripts of the *Xenopus* A6 somatic cell line. In Fig. 3, total early embryonic *Xenopus* transcripts are compared with transcripts from *Xenopus* A6 tissue culture cells. RNA species of low molecular weight from *Xenopus* tissue culture cells (Fig. 3, lane *3*) include the major ribosomal RNA species (a 5.8S doublet and 5S), cytoplasmic 7S RNA and tRNA. U2 is faintly visible, present in much lower amounts than these major transcripts. In the *Xenopus* embryo, however, after the onset of transcription, the major transcripts are snRNAs, tRNA, and a small amount of 7S RNA Fig. 3, lanes *1* and *2*). Since an approximately equivalent amount of labeled tRNA and 7S RNA was loaded in lanes *1–3* in Fig. 3, the relative rates of U2 synthesis in these two different *Xenopus* cell types can be compared. The autoradiograph in Fig. 3 was scanned with a densitometer. U2 values were normalized to labeled 7S and to tRNA, taking into account for the case of the embryo the different uridine content of U2 and 7S found in mammalian cells, 30% and 18%, respectively (37, 46). This type of internal normalization was necessary to take into account the different radioactive labels used ($^{32}$P-rUTP and $^{32}PO_4^-$). The difference observed between the amount of newly transcribed U2 in embryos and the tissue culture cells is 10- to 20-fold. With respect to this normalization, we cannot rigorously exclude the possibility that in the cultured cells, because of the labeling procedure, RNA polymerase II transcription of snRNAs was decreased relative to RNA polymerase III transcription of 7S and tRNA, making the normalization incorrect. However, this possibility seems unlikely since Johnson et al. (20) found less than a twofold change in the ratio of RNA pol II transcripts to RNA pol III transcripts between resting and growing 3T3 cells. Our estimation of high snRNA transcription in the embryo requires that embryonic tRNA transcription be equal or greater than that of *Xenopus* cultured cells. In support of this, Shiokawa et al. (42) found the rate of tRNA transcription in the *Xenopus* blastula to be 15 ng/embryo/h and to remain at approximately this level throughout the neurula stage, while

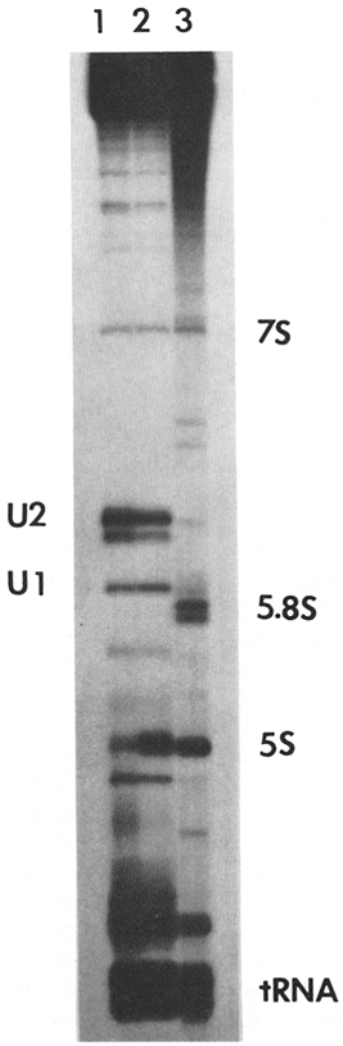


FIGURE 3 A Comparison of transcription products made in early *Xenopus* embryos with those of *Xenopus* tissue culture cells. Approximately equal amounts of labeled RNA (and DNA) from *Xenopus* embryos (lanes *1* and *2*; injected with $^{32}$P-rUTP at the one-cell stage and incubated for ~11 h), and *Xenopus* A6 tissue culture cells (lane *3*; labeled with $^{32}PO_4^-$) were separated on a 5% acrylamide 7 M urea gel at 1,500 V. Early embryonic transcripts were identified by size comparison with ribosomal RNA standards and from the immunoprecipitation results of Fig. 2.

Brown and Littna (5) found tRNA synthesis at blastula-neurula stages to be over 100-fold greater than at swimming tadpole stages. We would expect then that tRNA transcription in the rapidly growing embryonic stage measured here is at least as active as that in cultured cells and that the comparison of U2 synthesis is valid. The calculated 10- to 20-fold greater transcription of U2 RNA in the embryo is a minimum estimate of the observed difference. This difference suggests either that the rate of snRNA transcription is greater in the embryo than in tissue culture cells or that more embryonic U2 genes are active per cell in transcription.

The approximate number of newly transcribed RNA molecules could be calculated using data from the experiment in Fig. 1. For example, to calculate the number of new U1 molecules accumulated by 9 h after fertilization, the U1 band was cut out of the gel and counted (168 cpm). Since the amount and specific activity of $^{32}$P-rUTP injected at the one-cell stage ($5 \times 10^5$ cpm; 308 Ci/mmol), the pool size of rUTP at the relevant stages of development (1,000 pmol/egg; constant from 4.5 h; Kobayashi and Kirschner, unpublished data), and the size and approximate uridine-content of U1 (165 nucleotides; 26%) were known, the cpm in the U1 band could be converted into U1 molecules. The half-life of U1 RNA has been reported to be greater than 24 h (58), and decay of the newly transcribed RNA was considered to be negligible. The number of new U1 RNA molecules synthesized by 9 h was calculated to be $\simeq 5 \times 10^9$; the number synthesized by 8 h, $2.5 \times 10^9$. Thus, between 8 and 9 h synthesis of $2.5 \times 10^9$ molecules/h was observed or, dividing by the number of cells present ($\simeq$20,000), $\simeq 1 \times 10^5$/nucleus. (The cell number/embryo approximately doubles between 8 and 9 h) These calculations, in addition to providing a number value for the U1 molecules per nucleus in *Xenopus*, demonstrate the high rate of snRNA transcription in the embryonic cells, as already inferred in Fig. 3, above.

## *The SnRNA U1 Is Stored in Large Amounts in the Transcriptionally Inactive Embryo and Increases Significantly after the Onset of Transcription*

To determine whether snRNA is present in the egg and whether snRNA transcription significantly alters the total amount of snRNA present in the embryo, the U1 RNA isolated from embryos of different developmental stages was quantitated. RNA extracted from equal numbers of embryos was fractionated on a 10% polyacrylamide, non-urea gel and transferred to DBM paper. The blot was then hybridized with a $^{32}$P-labeled human U1 probe. An autoradiograph of the blot can be seen in Fig. 4 *A*, with the lanes containing embryonic RNA labeled with the time after fertilization. As shown in Fig. 4 *A*, a doublet of U1 RNA is present in all stages of embryonic development (Fig. 4, lanes *d–i*) and is also present in mature and immature oocytes (Fig. 4, lanes *a–c*). We do not know whether the U1 doublet corresponds to the one seen on urea-containing gels (Fig. 2) or whether the doublet represents U1 RNA and a degradation product of U1 RNA. Nuclear RNA from *Xenopus* liver was simultaneously probed for U1 content as a control, shown in lane *j* (Fig. 4).

As can be seen from the autoradiograph, U1 is present in the oocyte and early cleavage stage embryo (Fig. 4, lanes *a–f*). However, the total amount of U1 per embryo increases significantly as development proceeds. To determine more accurately the amount of U1 RNA present at each embryonic stage, the autoradiograph in Fig. 4 was scanned with a densitometer, the blot rehybridized with a labeled 28S rRNA probe, and the resulting autoradiograph scanned for 28S RNA content per lane. The U1 values obtained from the first autoradiograph were then normalized to 28S RNA content, which does not change in the developmental stages analyzed (6, 41), and graphed in Fig. 4*B*. The amount of U1 RNA present in the embryo at 4 h after fertilization (before the onset of transcription) is defined as one "egg equivalent." The U1 content of stage six oocytes and one-cell embryos derived from this analysis proved to be 0.5 instead of 1 egg equivalent, but it is not possible to distinguish between scatter of the data and a slight amount of early transcription. (The nucleic acid from these two developmental time points, because of the high yolk content, had to be extracted with phenol many more times than the other time points. We believe that this led to the lower amounts of U1 RNA seen in Fig. 4*A* at these times.) At later stages in development (8.75, 9.5, and 11 h after fertilization), a dramatic increase is seen in the total amount of U1 in the embryo, sevenfold at 11 h. The number of nuclei in the embryo is increasing at approximately the same rate as snRNA transcription, suggesting a coupling of the two.

The amount of U1 RNA present in the egg can be calculated from the above data by using the number of newly synthesized U1 molecules present at $\simeq$9–11 h ($5 \times 10^9$) and dividing by 6

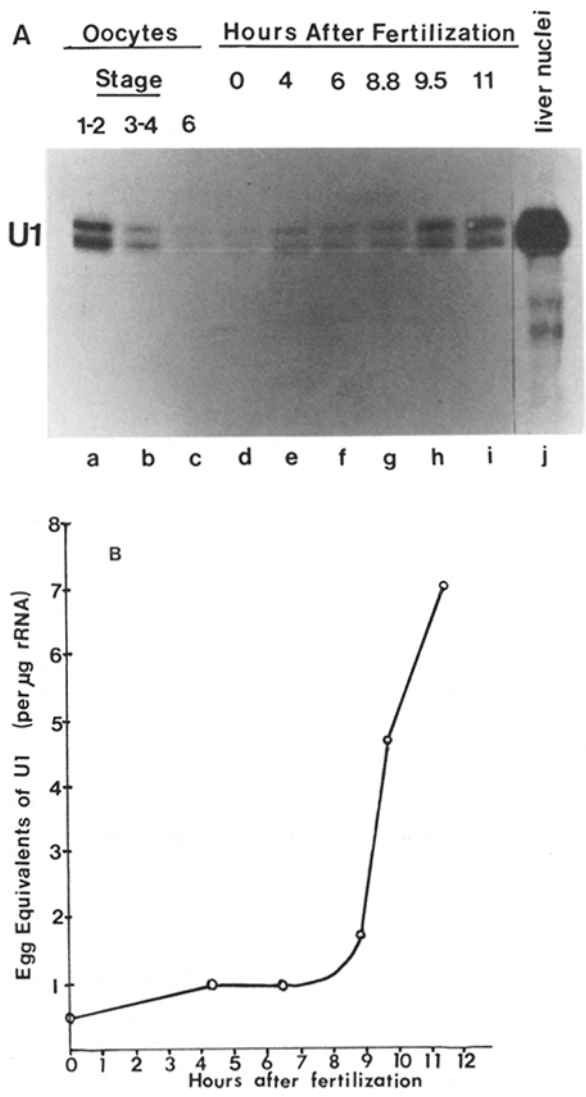


FIGURE 4 Early embryonic transcription increases the total amount of U1 small nuclear RNA present in the embryo. (*A*) Hybridization analysis. RNA (and DNA) extracted from approximately 3.75 embryos of different developmental stages were separated on a 10% acrylamide gel and transferred to DBM paper. The resulting blot was hybridized to labeled human U1 cloned DNA (≃$10^6$ cpm). The embryonic stages examined, expressed as age after fertilization, were: 0.5 h (one-cell stage), 4.25 h (sixth to seventh cleavage), 6.25 h (ninth to eleventh cleavage), 8.75 h (thirteenth to sixteenth cleavage), 9.5 h (pigmented crescent present, blastopore started), and 11.25 h (complete blastopore formed; approximately Nieuwkoop-Faber stage 10 1/2 [30]). RNA from 3.75 large (stage 6) oocytes was also examined in lane c. Lanes a and *b* show RNA from a larger and undetermined number of stage *1–2* and stage *3–4* oocytes. Low molecular weight nuclear RNA from *Xenopus* liver is shown in lane *j*. (*B*) Quantitation of the amount of U1 per embryo at different stages. The autoradiograph in *A* was scanned with a Zeineh densitometer. The blot was then rehybridized with a labeled *Xenopus* 28S ribosomal DNA probe and reexposed for autoradiography. This autoradiograph was scanned and the signal in *A* normalized to the amount of ribosomal RNA present per sample (since the amount of ribosomal RNA changes very little in these embryonic stages [6, 41]). This amount of U1, present in embryos prior to the onset of new transcription (4 and 6 h after fertilization), was defined as one egg equivalent.

to give $8 \times 10^8$ stored U1 molecules or enough for around 8,000 nuclei. The value for stored U1 molecules in the egg was confirmed by comparison of the amount of U1 per egg to that present in A6 cells (Northern blot not shown). An amount ≥8,000 A6 cells' worth of U1 was found per egg (assaying several individual eggs from different frogs extracted in several ways). It is worth noting here that the embryo has 4,000 cells at the time transcription first turns on. These experiments clearly demonstrate that the egg contains a store of snRNA sufficient for 4,000–8,000 nuclei. New embryonic transcription results in a major increase in the total amount of at least one snRNA (U1) in the embryo, and this increase correlates with the increase in the number of nuclei at this stage in development.

## *Xenopus Eggs Contain SnRNP Proteins and Have the Capacity to Assemble Exogenous SnRNA*

Many cellular components, including histones and ribosomes, have been found to be stored in excess in the early embryo (23, 50, 56). The hybridization analysis above provided evidence that the snRNA U1 is stored in the transcriptionally inactive early embryo. To determine whether the proteins normally associated with snRNA in snRNP particles are also present prior to the onset of transcription, *Xenopus* fertilized eggs were injected with radioactive snRNA species purified from total labeled embryonic RNA on gels, allowed to incubate for several hours, then lysed and subjected to immunoprecipitation with anti-snRNP antisera. Immunoprecipitation of the RNA would imply not only the presence of protein antigens in the egg but also their assembly onto the injected snRNA.

When anti-RNP antiserum was used, only U1 was immunoprecipitated (Fig. 5, lane *b*), whereas when anti-Sm-RNP antiserum was used, both U1 and U2 were precipitated (Fig. 5, lane *c*). U1 was quantitatively precipitated from the extract as the supernatants show no U1 remaining (Fig. 5, lanes *b'* and *c'*). Immunoprecipitation of U2 was only partial (Fig. 5, lane *c'*). Limiting antibody or assembly of the antigenic protein with only a fraction of the injected U2 molecules may have caused the partial precipitation. In all such immunoprecipitations, U1 was readily immunoprecipitable, while U2 was less so. If antibody was omitted from the immunoprecipitation procedure, neither U1 nor U2 was precipitated (Fig. 5, lane *a*) but were left in the supernatant (Fig. 5, lane *a'*). The RNA molecules themselves are not antigenic (25), the antigens having been shown to be proteins (17, 25, 32, 51, 52); therefore, the antigenic proteins must be associating with the injected snRNA. Preliminary results indicate the association is rapid, occurring <20 min after injection of the RNA. It can be concluded that the Sm and RNP antigenic proteins are present in the egg and can be readily assembled onto *Xenopus* U1 and U2 RNA, when the RNA is injected into fertilized eggs. In addition, since the amount of RNA injected in the experiment shown in Fig. 5 was approximately two unfertilized eggs' worth of U1 RNA, an excess of snRNP protein over snRNA is implied.

## *Xenopus SnRNP Proteins Can Assemble onto U1 RNA Molecules Differing Greatly in Sequence*

We wished to know whether the *Xenopus* snRNP protein(s) recognize the sequence or secondary structure of the injected

RNA. Because the sequence of *Xenopus* U1 is not known and thus could not be used in a comparison of assembly of two snRNAs differing in sequence, mouse and *Drosophila* U1 (and U2) were used. The sequence of mouse U1 RNA differs from that of *Drosophila* U1 by 30%, although they appear to form identical secondary structures (27). Mouse RNA of the size range of *Xenopus* U1 and U2 (Fig. 6*A*, lane *e*) was extracted from a urea gel. The U1- and U2-sized RNAs were mixed and injected into fertilized *Xenopus* eggs. Immunoprecipitation and analysis of the RNA in the precipitates and supernatants was performed as described for injected *Xenopus* snRNA. Anti-RNP antiserum precipitated only U1 and a molecule similar in size to the *Xenopus* U1 fragment seen in Fig. 2 (Fig. 6*A*, lane *b*). Anti-Sm-RNP antiserum precipitated U1 and, to a lesser extent, U2 (Fig. 6*A*, lane *c*) as in the *Xenopus* snRNA injections. A faint U1 band is visible in the control lane without antibody and may indicate U1 or labeled 5.8S RNA (which runs with U1 on these gels), binding nonspecifically to the Staph A immunoadsorbant (Fig. 6*A*, lane *c*). (As can be seen in the supernatants resolved in Fig. 6*A*, lanes *b'*, *c'*, and *d'*, the injected RNA was either partially ligated by the RNA ligase responsible for tRNA processing, known to be present in *Xenopus* [15] or another undescribed ligase activity. It is also possible, although unlikely, that the RNA is aggregated into higher molecular weight forms).

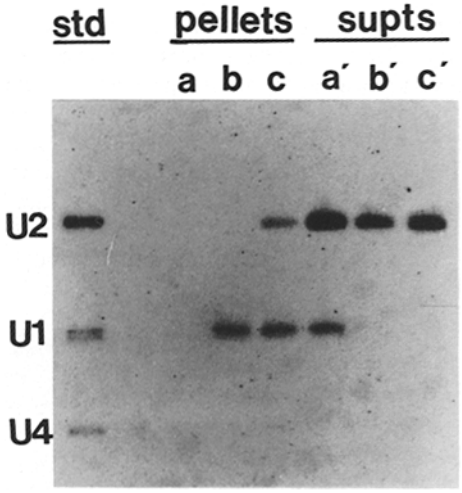


FIGURE 5 Fertilized eggs contain snRNP proteins—immunoprecipitation of *Xenopus* U1 and U2 RNA after injection into fertilized eggs. $^{32}$P-in vivo labeled RNA from labeled *Xenopus* embryos was separated on a 5% acrylamide 7 M urea gel. Radioactive U1 and U2 were extracted from the gel and injected into 16 fertilized *Xenopus* embryos at the one-cell stage. After 3 h, the embryos were lysed in 750 μl of cold buffer, and divided in three parts, after removal of yolk by centrifugation. Immunoprecipitation with 5 μl of anti-RNP was performed on one part (lanes *b* and *b'*), 5 μl of anti-Sm-RNP on a second (lanes c and c'), and no antiserum was added to a third (lanes *a* and *a'*). RNA from the immunoprecipitated pellets is shown in lanes *a*–*c* and the supernatants of these precipitations in lanes *a'*–*c'* (separated on a 5% acrylamide 7 M urea gel). In vivo snRNA size markers obtained from labeled *Xenopus* embryos are shown in the far left lane.

When *Drosophila* U1 and U2 were injected into fertilized frog eggs, a similar result was obtained (Fig. 6*B*). The injected RNA is shown in lane *f* (Fig. 6*B*). Anti-RNP antiserum (Fig. 6*B*, lane *c*) immunoprecipitated a band slightly smaller than *Xenopus* U1 (Fig. 6*B*, lane *b*) and identical in size to a prominent RNA species present in labeled *Drosophila* cultured cell RNA (Fig. 6*B*, lane *a*). Anti-Sm-RNP antiserum precipitated this band and a band identical in size to *Xenopus* U2 (Fig. 6*B*, lane *d*). The U2-sized band is also identical to a prominent RNA species seen in labeled *Drosophila* RNA (Fig. 6*B*, lane *b*). Although we assumed that the injected RNAs were *Drosophila* U1 and U2 because of their prominence and size, the immunoprecipitation results confirm this. As before, *Drosophila* U1 is precipitated quantitatively, while *Drosophila*

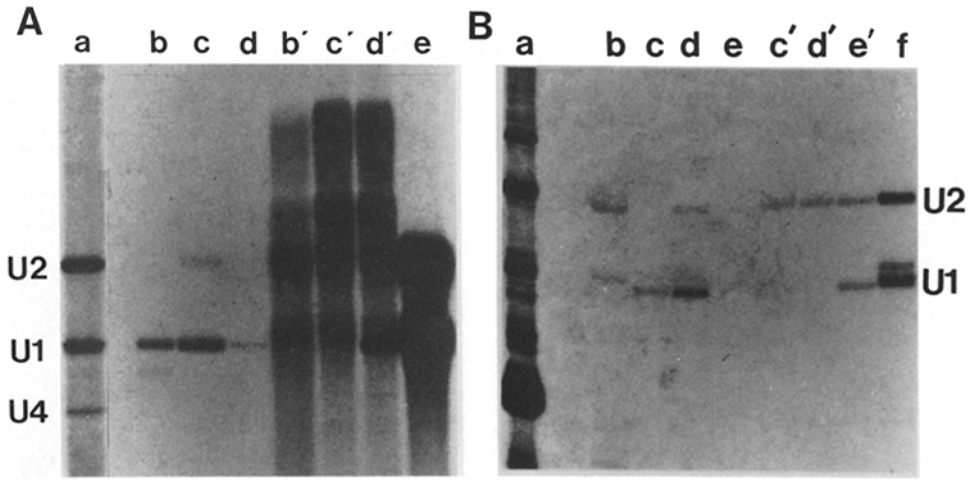


FIGURE 6 Assembly of mouse and *Drosophila* snRNA into immunoprecipitable entities following injection into fertilized *Xenopus* eggs. (*A*) Mouse U1 and U2. RNA was isolated from $^{32}PO_4^-$-labeled mouse S49 cells and separated on an acrylamide-urea gel. RNAs approximately the size of U1 and U2 were extracted from the gel. Approximately 3,000 cpm of mixed U1- and U2-sized RNA were injected into 15 fertilized *Xenopus* eggs. After 3 h, the injected eggs were lysed, immunoprecipitated, and analyzed as described in Fig. 5. An autoradiograph of the gel shows the RNA precipitated by anti-RNP antiserum (lane *b*), by anti-Sm-RNP (lane c), and in the absence of antibody (lane *d*). The RNA remaining in the supernatants of the immunoprecipitations is shown in lanes *b'*, *c'*, and *d'*, respectively. Lane e contains a sample of U1- and U2-sized mouse RNA prior to injection. Lane *a* contains labeled *Xenopus* embryonic RNA. It should be noted that mouse 5.8S runs with the same mobility as U1 and, as it probably represents the majority of U1-sized mouse RNA injected, may account for the faint U1-like band seen in the no antibody control. (*B*) *Drosophila* U1 and U2. Discrete RNA species the size of *Xenopus* U1 and U2 were obtained from *Drosophila* Kc tissue culture cells labeled with $^{32}PO_4^-$ in a manner similar to that described in *A*. Approximately 1,400 cpm of *Drosophila* U1 and U2 RNA were injected together into 15 fertilized *Xenopus* eggs, immunoprecipitated, and analyzed as in Fig. 5. An autoradiograph shows the immunoprecipitated RNA obtained with anti-RNP (lane c), that with anti-Sm-RNP (lane *d*), and that when no antibody is present (lane e). The RNA remaining in the supernatants of these immunoprecipitations is shown in lanes *c'*, *d'*, and *e'*, respectively. Lane *a* shows the pattern of labeled *Drosophila* tissue culture cell RNA in the U1 and U2 size range, lane *b* labeled *Xenopus* embryonic RNA (U2 and U1), and lane *f* a sample of *Drosophila* U1 and U2 prior to injection.

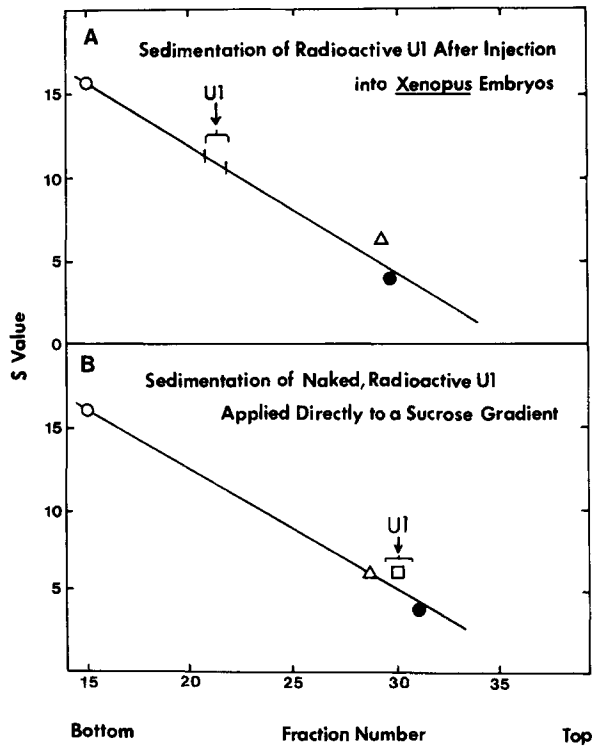


FIGURE 7 Sedimentation coefficient of radioactive U1 following injection into *Xenopus* fertilized eggs and a comparison with naked U1 RNA. (*A*) Labeled *Xenopus* U1 RNA was injected into 20 eggs, incubated for 3 h; and the embryos lysed and fractionated on a 12-ml 15–30% sucrose gradient. Labeled tRNA and 5S RNA markers, β-galactosidase (16S), and alkaline phosphatase (6.2S) were mixed in with the extract before addition to the sucrose gradient. Fractions were collected and analyzed as described in Materials and Methods. The S value of U1 following injection, determined with reference to the internal standards, is shown. The standards were *E. coli* β-galactosidase (○), alkaline phosphatase (△), 5.8S RNA (□), and tRNA (●). (*B*) Isolated radioactive U1 was added with the same S value markers as in *A* to a parallel sucrose gradient. The S value of isolated U1 is shown.

U2 is only partially immunoprecipitated. These experiments demonstrate that *Xenopus* snRNP proteins can assemble onto the snRNA from species as distantly related as mouse and *Drosophila*. More importantly, since the sequences of mouse and *Drosophila* U1 snRNA differ by 30%, these results suggest that the structure of the RNA plays a greater role in assembly of the snRNP protein(s) onto the RNA than the sequence itself, consistent with the studies of Mount and Steitz (27) showing that theoretically mouse and *Drosophila* U1 can fold into an identical secondary structure.

### *SnRNP Particles Formed with Injected SnRNA Resemble Native SnRNP Particles*

To assess whether the immunoprecipitable entities formed upon injection of snRNA into *Xenopus* eggs are structurally similar to in vivo snRNP particles, two types of experiments were performed. In the first, the sedimentation coefficient of U1 before and after injection was measured. SnRNPs in vivo have an S value which has been previously reported as 10S-11S (8, 27), whereas naked U1 RNA has an S value of ≈6S. Radioactive U1 fractionated 3 h after injection into embryos had an S value of 11S (Fig. 7). Uninjected radioactive U1 sedimented at 5S–6S, as expected. Thus, injection of U1 raised the S value of U1 RNA to that of in vivo snRNP particles.

In the second type of experiment, the secondary structure of the snRNA in the particle was probed with RNAse and compared to that in in vivo snRNP particles. Epstein et al. (18) have previously demonstrated that T1 RNAse digestion of HeLa cell snRNP particles produces specific nicks, presumably at sites unprotected by snRNP proteins, and that, upon immunoprecipitation, specific fragments are obtained. To examine the result of T1 RNAse treatment of *Xenopus* snRNP particles, embryos were injected with $\alpha$-$^{32}$P-rUTP and allowed to develop past the onset of transcription. The embryos were then lysed, T1 RNAse was added for 30 min, and immunoprecipitation performed with anti-RNP antiserum. The U1 fragments resulting from T1 digestion are shown in Fig. 8*A* in the lane designated "+T1." Five main fragments are visible: a major fragment slightly smaller in size than a U5 size marker (117 nucleotides), a second major fragment the size of the smallest of the tRNA size markers, and three much smaller fragments. When T1 is omitted from the procedure (Fig. 8*A*, "−T1"), a few minor bands appear in addition to the intact U1 band, but none of these correspond to those resulting from T1 digestion. In contrast (Fig. 8*C*), a partial T1 digest of naked U1 RNA gives three major fragments, at least two of which are not the size of those found in snRNP particles. To determine which T1 digestion products are obtained after digestion of injected U1 RNA, a similar protocol was followed. Fertilized eggs were injected with radioactive *Xenopus* U1. After incubation of the injected embryos for 3 h, T1 digestion, immunoprecipitation, and RNA extraction were performed. U1 fragments of a size identical to those shown in the Fig. 8*A* result (Fig. 8*B*, "+T1"). Thus, when T1 RNAse is used as a probe, the snRNP particles formed with injected U1 appear identical to those formed in vivo. This finding, together with the sedimentation coefficient results above, suggests that injected U1 snRNA becomes associated in a normal manner with the proteins bound to snRNAs in vivo.

## DISCUSSION

Following fertilization, the early cleavage period of the *Xenopus* embryo is characterized by rapid, synchronous cell division without concurrent transcription or cell growth. This period abruptly terminates after 12 cleavages (4,000 cells; the midblastula stage). When transcription is turned on, snRNAs were found to be major RNA polymerase II transcripts (Figs. 1 and 2). Five snRNA species, corresponding in size to U1, U2, U4, U5, and U6, were identified by immunoprecipitation from transcribing *Xenopus* embryos, a result identical to that seen with HeLa cells (26). We find that the only newly synthesized RNA species, other than snRNAs, in any abundance are RNAs that migrate with 7S RNA and tRNAs.

Newly transcribed embryonic RNA was compared to *Xenopus* cultured cell RNA. In RNA samples containing approximately equal amounts of labeled tRNA and 7S RNA, the lower molecular weight RNA species in embryos were found to be primarily 7S RNA, tRNA, and snRNA and, in cultured cells, to be 7S, 5.8S, 5S, tRNA, and the snRNA U2. When normalized to tRNA and 7S RNA, the amount of labeled U2 was much higher in the embryo than in cultured cells (≃10- to 20-fold in Fig. 3). Thus, the data are consistent with a greater rate of snRNA transcription in the embryo or a greater number of snRNA genes active in embryonic transcription, a

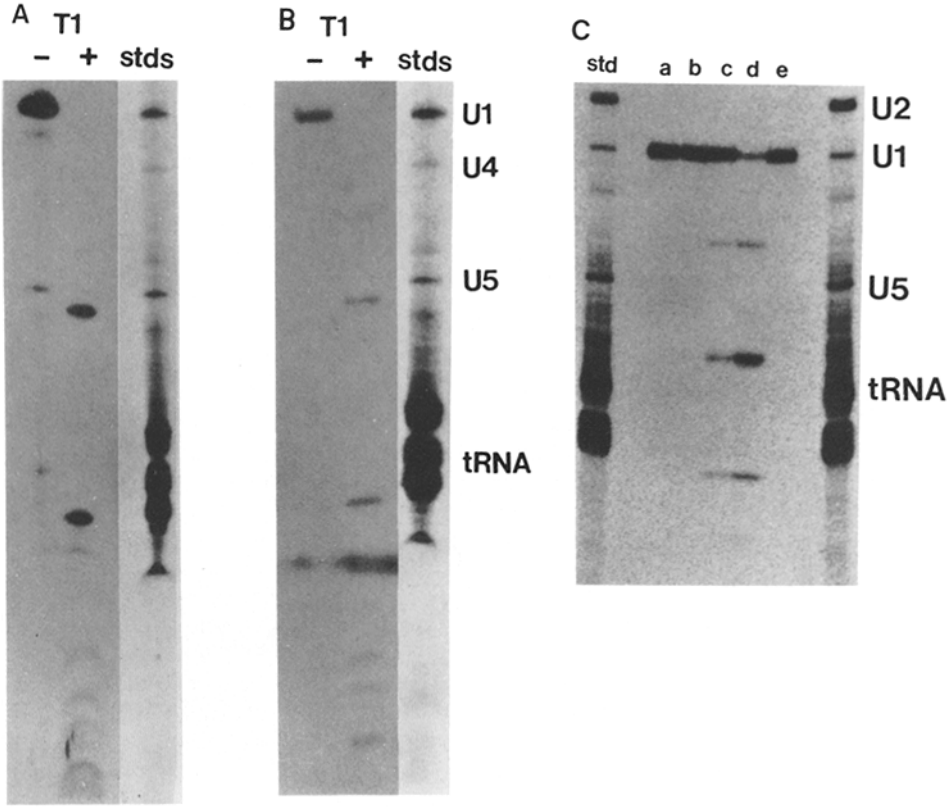


FIGURE 8 A comparison by T1 RNAse digestion of in vivo U1 snRNP particles with the U1 snRNP particles assembled after injection of U1 RNA. (*A*) T1 digestion of in vivo U1 snRNP particles. 10 fertilized *Xenopus* eggs were injected with $\alpha$-$^{32}$P-rUTP, allowed to develop for 10 h, and lysed in buffer. After removal of the yolk, the embryo extract was split into two parts, and 30 $\mu$g of T1 RNAse was added to one part. Both parts were incubated at 0°C for 30 min, at which time anti-RNP antiserum was added. Immunoprecipitation was performed and the RNA present in both precipitate and supernatant was analyzed as described in Materials and Methods. The RNA immunoprecipitated by anti-RNP from in vivo $^{32}$P-labeled embryos in the presence (+) and absence (−) of T1 RNAse treatment is shown. Total labeled *Xenopus* embryonic RNA is provided for size markers. (*B*) T1 digestion of U1 snRNP-like particles formed after injection of radioactive U1. In vivo labeled *Xenopus* U1 was injected into 10 eggs, allowed to incubate for several hours, and treated exactly as in *A*, one-half being digested with T1 RNAse prior to immunoprecipitation with anti-RNP antiserum. The RNA immunoprecipitated from embryos (injected with $^{32}$P-labeled *Xenopus* U1) in the presence (+) and absence (−) of T1 is shown. Total labeled *Xenopus* embryonic RNA is shown for size markers. (*C*) T1 digestion of naked U1 RNA. As described in Materials and Methods, naked U1 RNA was digested with 1.28 ng (lane *a*), 6.4 ng (lane *b*), 32 ng (lane c), and 160 ng (lane *d*) of T1 RNAse. U1 RNA carried through a similar procedure without T1 RNAse is shown in lane e. Labeled *Xenopus* embryonic RNA is shown in the side lanes for size markers.

result which raises the question as to whether there is blastula-specific snRNA transcription, in analogy with the oocyte-specific 5S synthesis seen in *Xenopus* (7, 47).

By hybridization of embryonic RNA with a cloned U1 probe (Fig. 4), the snRNA molecule U1 was found to be present in the embryo before the onset of transcription. From the same analysis, it could be determined that new embryonic snRNA transcription increased the total amount of U1 in the embryo approximately sevenfold by 4 h after the onset of transcription. This result, coupled with a calculation of the number of newly transcribed U1 molecules at 9 h ($\simeq 5 \times 10^9$), made possible a calculation of the number of U1 molecules stored in the egg: $\simeq 8 \times 10^8$. If the total number of stored U1 molecules is divided by the number of cells (4,000) present at the time when transcription is first turned on, a value for molecules of U1 per cell is obtained: $\simeq 2 \times 10^5$. This value is strikingly similar to the number of molecules transcribed per cell after embryonic transcription is turned on: $1 \times 10^5$. It would appear then that, despite a capability for very rapid synthesis of U1 at the 4,000-cell stage, the egg contains a store of U1 molecules sufficient to reach this developmental stage. Hybridization analysis comparing the amount of U1 present in the unfertilized egg to that in A6 cells confirmed this conclusion. (This result differs strikingly from those of Zeller et al. [58], but we believe our estimates, derived both from determination of the actual number of molecules present and from Northern blot comparisons of the amount of U1 RNA in embryos and *Xenopus* A6 somatic cells, to be correct, their estimate being based on the less accurate technique of dot blot analysis.) Storage of U1 in the transcriptionally inactive early embryo raises an interesting question as to the function of U1 at these early times. It is possible that a very low level of transcription is taking place, requiring the presence of U1 for processing or, alternately, U1-RNP particles may be fundamental structural components of all nuclei, whether transcribing or not. Since all transcription can be blocked in the early cleavage embryo by injection of $\alpha$-amanitin (28), it should be possible to address such questions of U1-RNP localization and function in future work.

Like the snRNA U1, snRNP proteins were also found to be present in the early *Xenopus* embryo, as assayed by injection of radioactive *Xenopus* U1 and U2 RNA into fertilized eggs and subsequent immunoprecipitation. Because the anti-snRNP antisera used is specific for protein components of snRNP particles, immunoprecipitation of the RNA indicates that snRNP proteins have complexed with the injected U1 and U2 RNA molecules. All of the injected U1 is immunoprecipitable, indicating that more snRNP proteins than snRNA are present. We have not yet determined the amount of injected U1 required to titrate the embryonic snRNP proteins; however, enough proteins are present in a single egg to confer antigenicity on the U1 snRNA isolated from $\simeq$16,000 *Xenopus* tissue culture cells (D. J. Forbes, unpublished results). This excess of snRNP proteins over snRNA is in agreement with the results of Zeller et al. (58). An excess of snRNP proteins over snRNA proteins in the egg would be available at the onset of transcription for the rapid assembly into snRNP particles of the large amount of newly transcribed snRNA described above.

By both T1 digestion pattern and S value, the U1 snRNP

particles formed after injection of U1 and the U1 snRNP particles isolated from in vivo labeled cells are identical. Surprisingly, *Xenopus* snRNP proteins also confer immunoprecipitability on mouse and *Drosophila* U1 and U2 snRNA. Although the sequence of *Xenopus* snRNA is unknown, mouse U1 RNA is only ≃72% homologous to that of *Drosophila* U1. However, mammalian and *Drosophila* U1 RNAs are theoretically able to assemble into the same secondary structure (27), and this may be the important element for recognition by *Xenopus* snRNP proteins. De Robertis et al. (16) have observed immunoprecipitation of HeLa snRNA by lupus anti-Sm antisera, following injection into mature *Xenopus* oocytes. They were able to show by autoradiography that the injected snRNA migrated into the germinal vesicle. Our data show that fertilized *Xenopus* eggs contain the proteins necessary to assemble *Xenopus* U1 into snRNP particles identical (by the critera used) to native particles. However, neither our data nor the nuclear association of De Robertis can exclude the possibility that certain snRNP proteins (which are nonantigenic ones) are absent from the immunoprecipitated particles. It is likely, though, that in vivo-like snRNP particles are formed following injection, and this should allow us to probe the cytological location of snRNA in the developing embryo and possibly perturb development by injection of excess snRNA.

The presence of large amounts of snRNA and snRNP proteins in the embryo before transcription turns on poses an interesting question: Is there a role for small nuclear RNAs in the nontranscribing nucleus? The answer to this question may not be the same for all five of the snRNAs in snRNP particles (U1, U2, U4, U5, and U6 [13]). In the early embryo, some of the snRNA species may migrate into the transcriptionally inactive nuclei, indicating a function not associated with transcription, while other snRNA species may remain in the cytoplasm, perhaps until transcription turns on at the midblastula stage. Preliminary results indicate that both the Sm and the RNP protein antigens are present in the nuclei as early as the 32-cell stage and increase at least 10-fold after the onset of snRNA transcription (D. J. Forbes, unpublished results). However, detection of snRNA in the early nuclei and differentiation between snRNA species may require in situ hybridization of embryonic tissue sections with cloned probes for snRNA. A gradual or abrupt migration of U1 into the nucleus around the time when transcription turns on could correlate, for example, with the observed 30% decrease in maternal RNA complexity seen at the midblastula stage, a decrease which might result from RNA degradation, or specific processing events (12).

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