

Sea Urchin Maternal and Embryonic U1 RNAs Are Spatially Segregated in Early Embryos

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Abstract. We have used in situ hybridization and cell fractionation methods to follow the distribution of U1 RNA and immunofluorescence microscopy to follow the distribution of snRNP proteins in oocytes, eggs, and embryos of several sea urchin species. U1 RNA and U1-specific snRNP antigens are concentrated in germinal vesicles of oocytes. Both appear to relocate after oocyte maturation because they are found primarily, if not exclusively, in the cytoplasm of mature unfertilized eggs. This cytoplasmic residence is maintained during early cleavage and U1 RNA is first detectable in nuclei of micromeres at the 16-cell stage.

Between morula and gastrula stages the steady-state concentrations of both RNA and antigens gradually increase in nuclei and decrease in cytoplasm. Surprisingly, analysis of the distribution of newly synthesized U1 RNA shows that it does not equilibrate with the maternal pool. Instead new transcripts are confined to nuclei, while cytoplasmic U1 RNAs are of maternal origin. This lack of equilibration and the conversion of maternal U1 RNAs from nuclear species in oocytes to cytoplasmic in embryos suggests that these RNPs (or RNAs) are structurally altered when released to the cytoplasm at oocyte maturation.

SMALL nuclear RNAs (snRNAs) play a key role in RNA biosynthesis in eukaryotic cells since they are essential for reactions in RNA processing, including splicing of mRNA precursors (Busch et al., 1982; Mount et al., 1983; Padgett et al., 1983; Kramer et al., 1984; Krainer and Maniatis, 1985). Maternal snRNPs are required during oogenesis for the processing of maternal mRNAs, and the most abundant small nuclear RNAs, U1 and U2, are present in large amounts in mature sea urchin eggs (Nijhawan and Marzluff, 1979; Brown et al., 1985). Additional U1 and U2 RNAs are apparently required for processing of embryonic transcripts synthesized after fertilization, because synthesis of these RNAs begins around the 32-cell stage and continues at high rates through cleavage, declining only after the rate of cell division decreases markedly (Nijhawan and Marzluff, 1979). This new synthesis results in an approximately threefold increase in steady-state U1 RNA concentration during embryogenesis (Brown et al., 1985).

In the experiments presented here we examine the localization of U1 RNA and snRNP antigens in sea urchin oocytes, eggs, and embryos using both cytological and biochemical techniques. We have compared the subcellular distribution of total U1 RNA to that of molecules newly synthesized after fertilization and have found that these two classes of molecules do not equilibrate.

Materials and Methods

RNA Probes

Two subclones of pLvU1.1 (Brown et al., 1985) were used to construct templates for in vitro transcription. A 110-base pair (bp) fragment extending from the Sau3A site at nucleotide 30 to the Sau3A (Bgl II) site at nucleotide 140 of U1 RNA was introduced into pSp64 and pSp65 in orientations yielding antisense or sense transcripts, respectively. A 400-bp fragment extending from 30 bases 5' of the U1 gene to the Dra I site 3' of the gene was constructed by digestion of pUID (Morris et al., 1986) with Bal 31 from the 5' end and was also cloned into the same pair of vectors. After truncation of these templates with either Eco RI (pSp64) or Hind III (pSp65), RNAs were synthesized with Sp6 RNA polymerase at a specific activity of 1.2×10^8 dpm/ μ g using 3 H-CTP and 3 H-UTP (28 and 40 Ci/mmol, respectively; Amersham Corp., Arlington Heights, IL) and purified as described by Cox et al. (1984). Probe concentrations used were sufficient to achieve saturation of available target RNAs and were 47 and 110 ng/ml, in proportion to probe complexity. Exposure times are given in the figure legends.

In Situ Hybridization

The in situ hybridization studies were carried out on ovaries, eggs, and embryos from both *Strongylocentrotus purpuratus* and *Lytechinus pictus*. All procedures for tissue fixation in 1% glutaraldehyde were as described previously (Angerer and Angerer, 1981). 1- μ m-thick sections, shown in Figs. 2 and 3, e and f, were cut with glass knives using an LKB ultramicrotome. Fixation, dehydration, and embedding times were doubled for the preparation of ovary tissue. In situ hybridization was carried out as described by Cox et al. (1984) with the following modifications. Prehybridization proteinase K digestion used concentrations between 1 and 5 μ g/ml, and hybridization temperatures were varied between 20° and 65°C in different experi-

ments as described below. Under our standard conditions (proteinase K digestion at 1 $\mu\text{g}/\text{ml}$ and hybridization at 45°C in 50% formamide, 0.3 M NaCl, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA for 16 h), we calculated from the observed grain densities, the known concentrations of U1 RNA ($\sim 10^7$ molecules/egg; Nijhawan and Marzluff, 1979) and signals obtained for sea urchin early histone and actin messenger RNAs present in embryos at known concentrations (Cox et al., 1984, 1986) that the efficiency of hybridization to U1 RNA was less than 1% of that we routinely achieve with probes for messenger RNAs. This is not surprising in view of the extensive secondary structure of U1 RNA and its tight complex with proteins, both of which undoubtedly reduce accessibility to hybridization probes, especially after glutaraldehyde fixation. It is also possible that U1 RNA is not retained efficiently in fixed tissue sections during the in situ hybridization procedure.

We tested a number of different conditions designed to maximize accessibility of U1 targets. These included treatment (alone and in some combinations) of sections of fixed tissue with higher proteinase K concentrations, with urea and SDS and with 20 mM HCl followed by incubation in Triton X-100 (Brigati et al., 1984) to help remove complexed proteins; incubation of sections in 0.015 M NaCl, 0.0015 M Na_3 citrate at 80°C to reduce secondary structure of U1 RNAs; and hybridization and washing at higher stringency (up to 65°C in hybridization buffer) to open secondary structure of both target and probe sequences, or at lower stringency (down to 20°C) to stabilize very short hybrid regions. None of these treatments resulted in significant increase in signal, but importantly they did not alter the relative hybridization levels in nuclei and cytoplasm or in embryos of different stages. Signals were only slightly higher for the longer probe, consistent with its greater sequence complexity, and patterns were identical to those obtained with the shorter probe. Because hybridization patterns were independent of a variety of experimental treatments and because the in situ data are in good agreement with both immunofluorescent detection of U1 RNP proteins and biochemical measurements, it is unlikely that preferential loss of U1 or differential accessibility can account for the observed patterns.

For the data presented in Fig. 3, random sections were photographed, and grain densities were determined from projected images as described previously (Angerer and Angerer, 1981).

Indirect Immunofluorescence Microscopy

Lytechinus variegatus and *S. purpuratus* eggs and embryos were fixed and treated with the primary antibody (1:100 dilution) followed by the fluorescein- or rhodamine-labeled second antibody, goat anti-human IgG, as described by Schatten et al. (1985). The primary antisera used were anti-RNP and anti-Sm (gifts from Dr. J. Steitz, Yale University), and an anti-Sm serum obtained from Dr. D. Price (Duke University). Background fluorescence due to primary or secondary antibodies alone was negligible.

Growth of Embryos and Cell Fractionation

L. variegatus were obtained at the FSU marine laboratory and embryos were raised at 25°C as previously described (Nijhawan and Marzluff, 1979). *S. purpuratus* were obtained from Marinus Inc. (Venice, CA), and embryos were cultured at 15°C. In all experiments >95% of the embryos developed normally. The embryos were separated into nuclear and cytoplasmic fractions by a modification of the procedure of Morris and Marzluff (1985). They were washed twice in 0.55 M KCl and once in 0.25 M sucrose, 10 mM EDTA, pH 8.0, and lysed by homogenization in 0.32 M sucrose containing 1 mM EDTA, 1 mM spermidine, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 10 mM Tris-HCl, pH 8.0. Nuclei were collected by centrifugation at 1,000 g for 10 min. Embryos were labeled with ^{32}P and RNA was isolated from the cytoplasmic and nuclear fractions as described previously (Nijhawan and Marzluff, 1979).

S1 Nuclease Assay for U1 RNA

The amount of U1 RNA was measured using a quantitative S1 nuclease assay as previously described (Brown et al., 1985). Cloned U1 gene DNA was cut with Bgl II at nucleotide 140, and the 5' end was labeled with γ - ^{32}P -ATP using T_4 polynucleotide kinase. The labeled DNA was hybridized with nuclear and cytoplasmic RNA. Typically 100 ng of cytoplasmic RNA and nuclear RNA from the same number of embryos were used. The S1-resistant DNA was analyzed by gel electrophoresis, detected by autoradiography, and quantified by densitometry.

Precipitation of snRNPs with Lupus Antisera

S. purpuratus eggs were collected in sea water and washed twice in 0.55

M KCl. 1 ml of eggs (approximately 10^6) were lysed by homogenization in 5 ml of 0.4 mM Tris, pH 7.5, 150 mM NaCl in a Dounce homogenizer (tight pestle). The homogenate was clarified by centrifugation for 10 min at 12,000 g. RNA was purified from an aliquot of both the pellet and supernatant. The remaining supernatant was divided into three portions and incubated for 15 min at 40°C with 10 μl of the appropriate antiserum. The control serum was anti-DNA serum obtained from the Communicable Disease Center, Atlanta, GA. The immunoprecipitates were collected with Pansorbin, washed, and RNA was prepared as described by Lerner and Steitz (1979).

Results

To determine the localization of the protein and RNA components of U1 RNP in eggs and early embryos we used three techniques: (a) in situ hybridization (Cox et al., 1984) to identify U1 RNA; (b) immunofluorescence microscopy using anti-RNP and anti-Sm sera to identify proteins associated with U1 RNPs; and (c) S1 nuclease assay of the U1 RNA content of cell fractions enriched for nuclei or cytoplasm. The cytological techniques were required because nuclei of unfertilized eggs, and probably also those of early cleavage stage embryos, are fragile and do not retain their RNA complements during standard cell fractionation procedures. For example, the large quantity of histone mRNA that is sequestered in egg pronuclei (DeLeon et al., 1983; Showman et al., 1983) is almost quantitatively lost to cytoplasmic fractions during standard procedures for nuclear isolation. Furthermore, sea urchins shed haploid eggs that have completed maturation some weeks before, and methods for isolation of pure populations of oocytes or for separation of germinal vesicles from cytoplasm are not available.

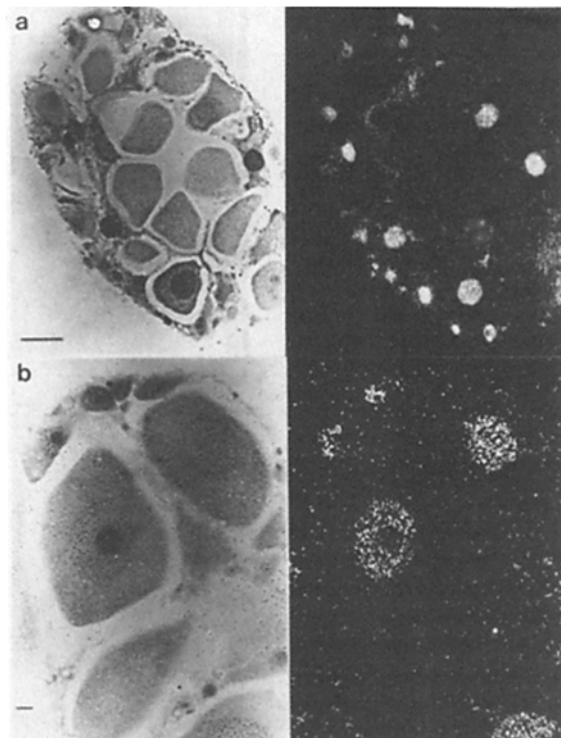


Figure 1. In situ hybridization to U1 RNA in ovary sections. ^3H -labeled RNA, complementary to nucleotides 30-140 of U1 RNA, was hybridized at 55°C to sections of *L. pictus* ovary that had been digested with 1 mg/ml proteinase K. Similar results were obtained with sections of *S. purpuratus* ovary (not shown). The same sections are shown in phase-contrast (left) and darkfield (right) illumination. Exposure time was 14 d. Bars: (a) 100 μm ; (b) 10 μm .

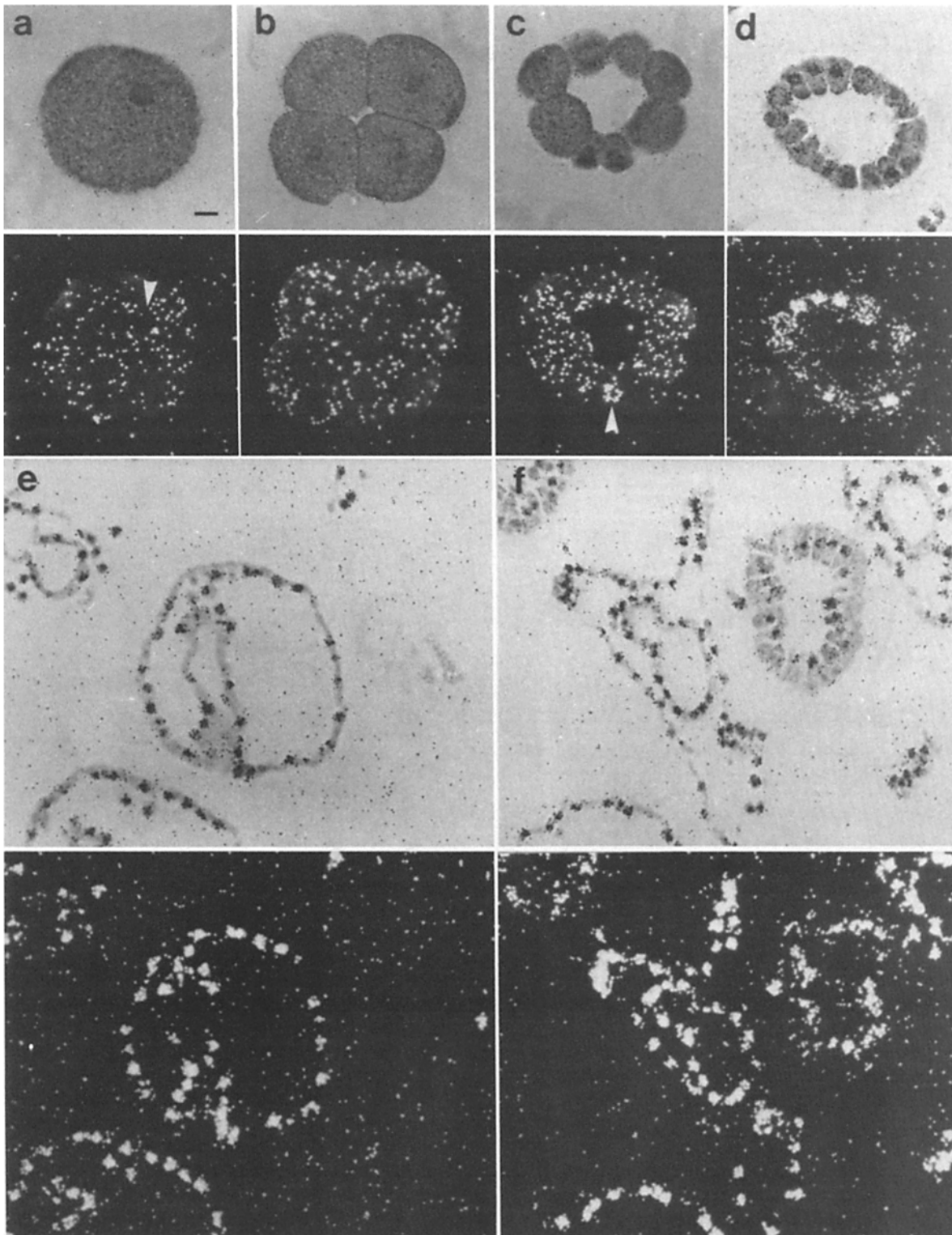


Figure 2. In situ hybridization to U1 RNA in eggs and developing embryos. ^3H -labeled antisense RNA was hybridized to sections of *S. purpuratus* eggs (a), and embryos at 4-cell (b), 16-cell (c), 12-h late cleavage (d), gastrula (e), and pluteus (f) stages. Individual sections shown were treated under slightly different conditions of proteinase K digestion or hybridization temperature but, as discussed in Materials and Methods, these variations did not affect signal magnitude or pattern. The same sections are shown in phase-contrast (top) and dark-field (bottom) illumination. The arrowhead in a indicates the egg pronucleus and that in c points to one of two micromeres visible in this section. Exposure times were 14 d (a, c, and d), 20 d (b), or 24 d (e and f). Bar, 10 μm .

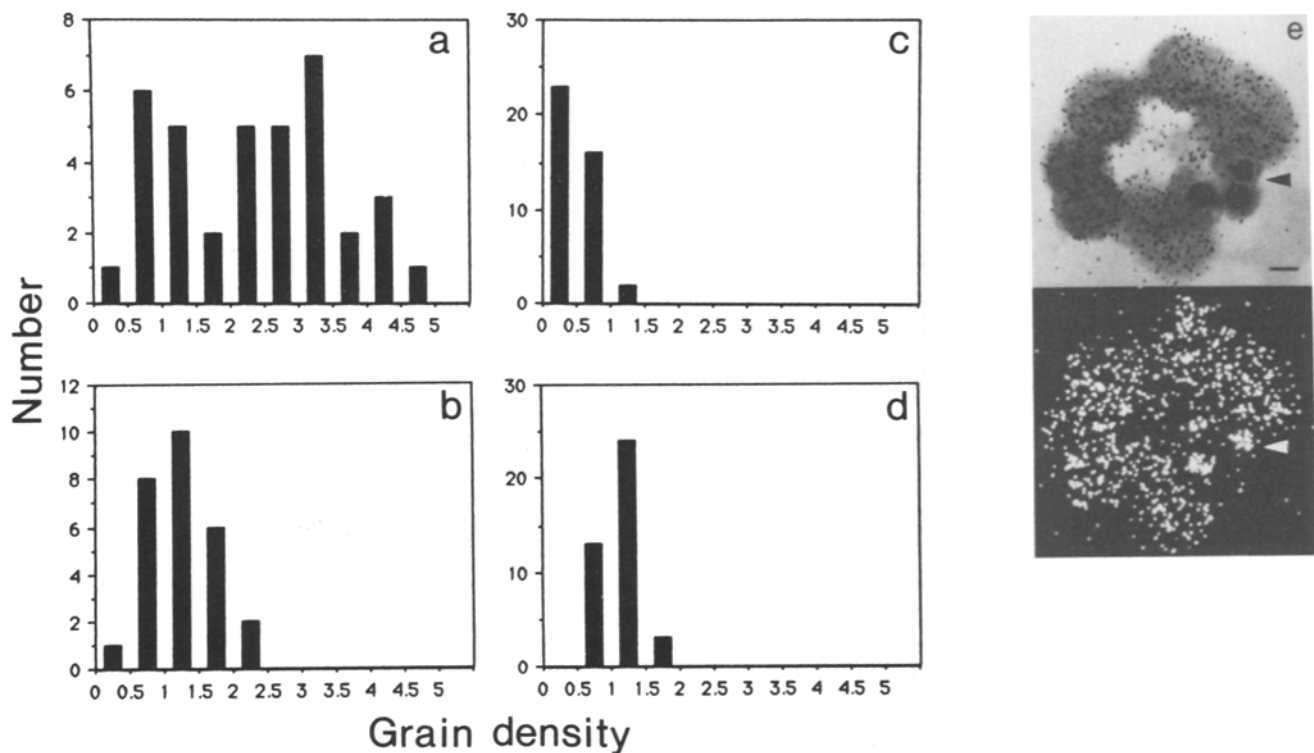


Figure 3. Comparison of nuclear and cytoplasmic grain densities in micromeres and other blastomeres. Grain densities (grains/10 μm^2) were determined as described in Materials and Methods. (a) Micromere nuclei; (b) nuclei of other blastomeres; (c) micromere cytoplasm; and (d) cytoplasm of other blastomeres. All values have been corrected for nonspecific probe binding, as measured in identical hybridizations with the sense strand probe. The micrographs shown in e illustrate the heterogeneity in labeling of micromere nuclei, and the lower signals observed over cytoplasm of micromeres (see arrowheads). Bar, 10 μm .

Localization of U1 RNA by In Situ Hybridization

We determined the distribution of U1 RNA directly by hybridizing ^3H -labeled antisense (signal) or sense (control) transcripts to tissue sections of ovaries, eggs, or embryos of the sea urchins, *S. purpuratus* and *L. pictus*. Two probes were used with essentially identical results for both sea urchin species. One probe consisted of nucleotides 30–140 of the *L. variegatus* U1.1 RNA coding region (Morris and Marzluff, 1985), and the other contained the entire gene plus some adjacent 5' (30 nt) and 3' (200 nt) sequence.

Fig. 1 a shows bright-field and dark-field photomicrographs of an ovary section hybridized with the shorter antisense ^3H -RNA probe. Labeling is found primarily over nuclei of both small and large oocytes. Fig. 1 b is a higher magnification micrograph that illustrates the absence of labeling over nucleoli and the large difference in U1 RNA concentration between nucleus and cytoplasm. In the largest oocytes we observed several-fold differences in nuclear grain densities, even over adjacent cells.

There is a dramatic shift in the subcellular distribution of U1 RNA to the cytoplasm after oocyte maturation. Haploid pronuclei are essentially unlabeled, as shown in Fig. 2 a. Determination of grain densities over cytoplasm of eggs hybridized with antisense and sense probes gave signal/noise ratios from 8 to 18 for different egg preparations and different experiments, while signals over nuclei were not distinguishable from background. We conclude that the 10^7 molecules of U1 RNA contained in the mature egg (Nijhawan and Marzluff, 1979; Brown et al., 1985) reside primarily, if not exclu-

sively, in the cytoplasm. This cytoplasmic localization is maintained throughout early cleavage (Fig. 2 b, 4-cell embryo).

Accumulation of U1 RNA in nuclei is not detectable until about 5 h after fertilization when the fourth cleavage divisions generate the 16-cell embryo consisting of eight animal pole mesomeres, four macromeres, and four micromeres located at the vegetal pole. At this stage, some micromere nuclei are labeled to a level that is about threefold higher than that of surrounding cytoplasm (Fig. 3, a, c, and e). In sections in which several micromere nuclei are clearly visible, usually not all of them are highly labeled. Conversely, U1 RNA concentrations are, on average, about two-fold lower in micromere cytoplasm than in the rest of the embryo (cf. Fig. 3 b and 3 d). We can exclude the simple model that the lower cytoplasmic content of micromeres results only from migration of U1 RNA into nuclei because cytoplasmic grain densities in individual micromeres are similar regardless of whether nuclei are labeled or unlabeled (data not shown). The grain densities over the less labeled micromere nuclei are equivalent to those over nucleus and cytoplasm of other blastomeres (Fig. 3, a, c, and d). Some micromere nuclei may appear less heavily labeled because of quenching of signals by overlying cytoplasm, although efforts were made to minimize this effect by analyzing only those sections in which nuclear borders were clearly defined. It is also possible that there are differences in timing of U1 RNA accumulation among the nuclei of different micromeres.

As development proceeds, labeling becomes progressively

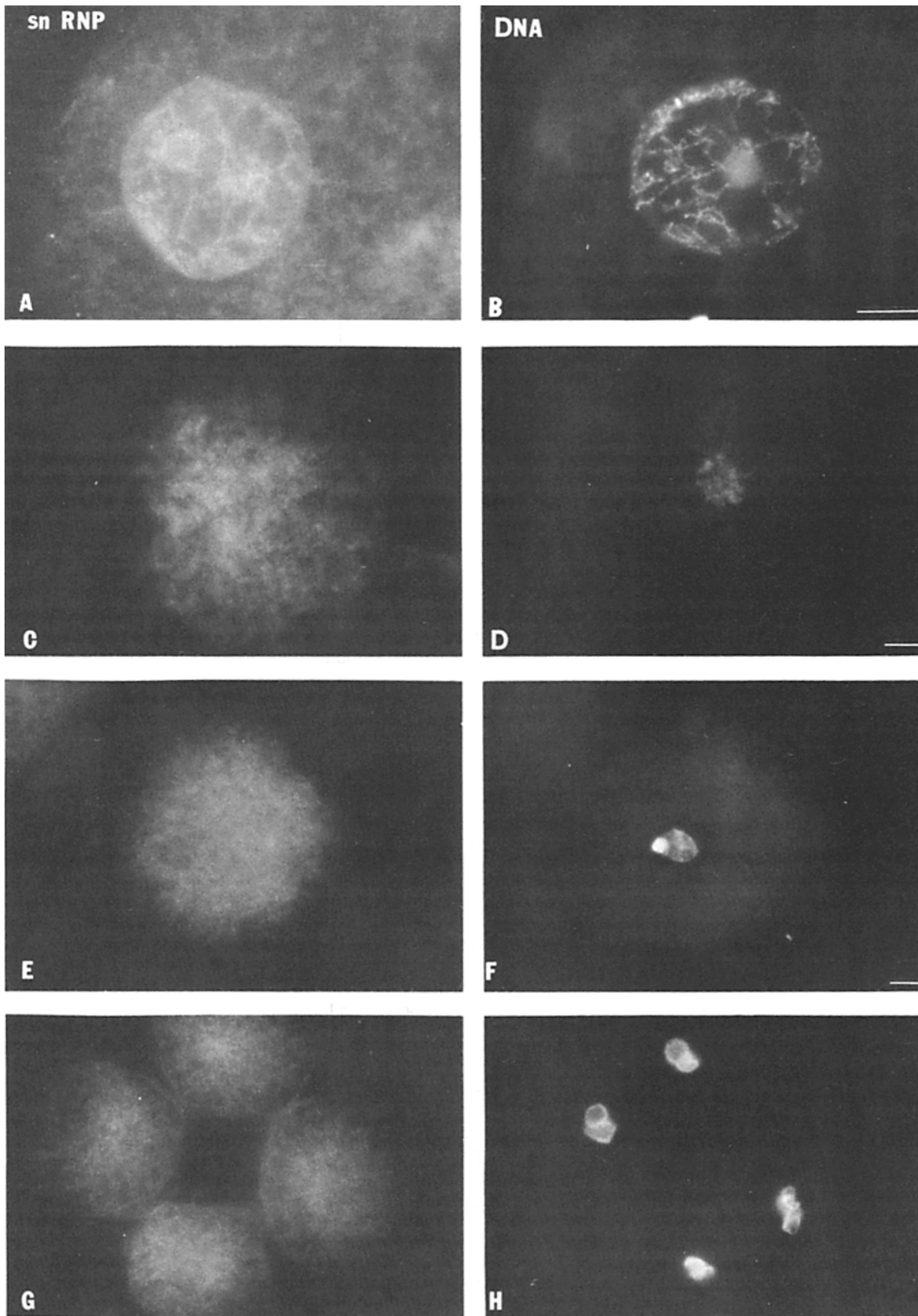


Figure 4. Localization of the RNP antigen in eggs and cleaving embryos of *L. variegatus* by immunofluorescence microscopy. Anti-RNP serum stains the germinal vesicle of an oocyte (A). There is also some cytoplasmic staining and the nucleolus is outlined with this serum. In unfertilized (C) and fertilized (E) eggs and during the first cleavages (4-cell stage: G), the nuclear staining is lost and instead only cytoplasmic fluorescence is observed. In B, C, F, and H, DNA fluorescence with Hoechst 33258 dye is shown. B and D are of oocytes and eggs corresponding to A and C, respectively, and F and H are double-stained images of E and G, respectively. Bar, 10 μ m.

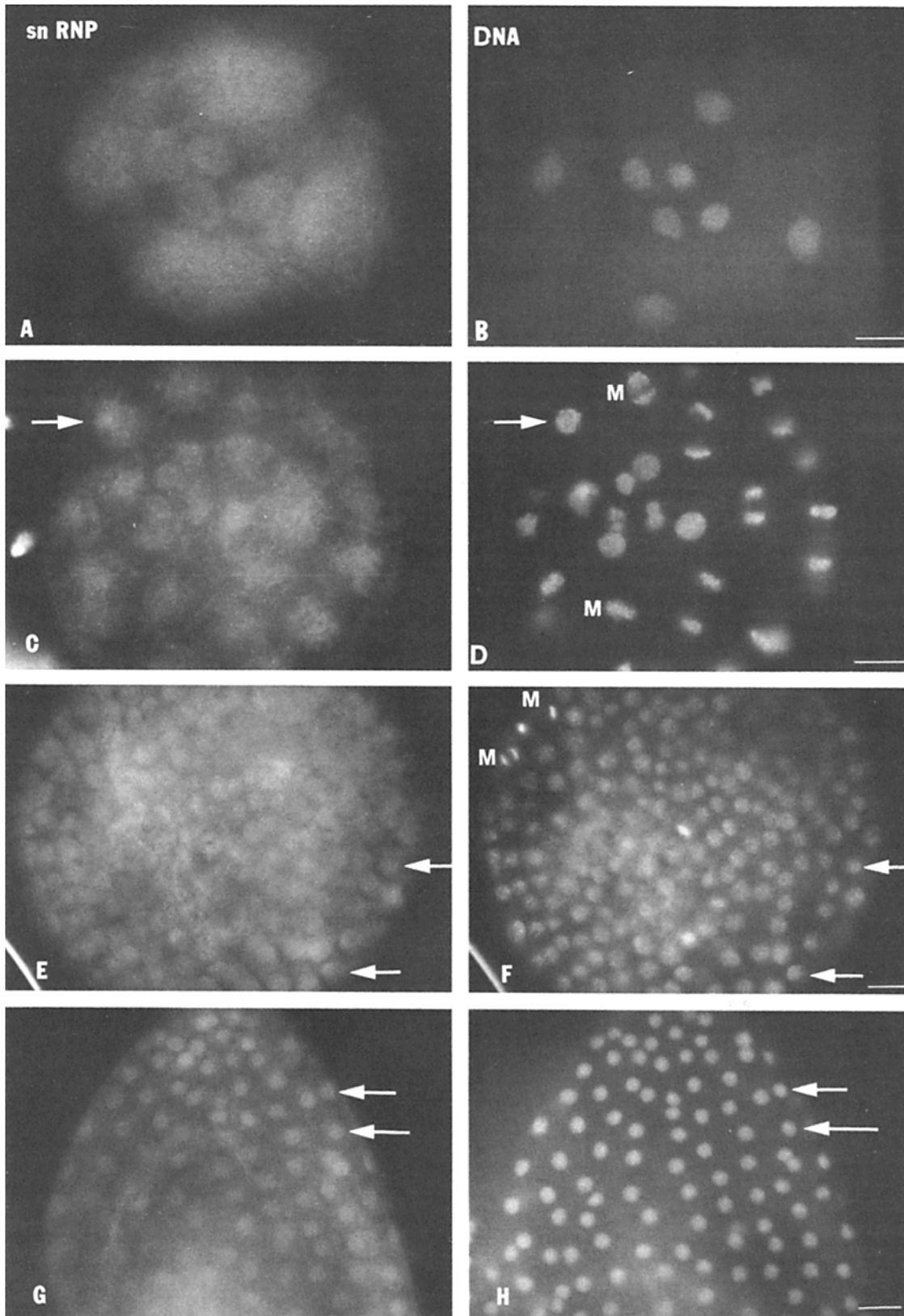


Figure 5. Localization of the RNP antigen in *S. purpuratus* embryos. 16-cell embryos, showing the four micromeres (*A* and *B*), early blastula (*C* and *D*) hatching blastula (*E* and *F*), and gastrula (*G* and *H*) stage embryos are shown stained with anti-RNP serum (*A*, *C*, *E*, and *G*) or DAPI (*B*, *D*, *F*, and *H*). The arrows point to corresponding nuclei in each embryo. The cells labeled *M* are in mitosis. Similar results were obtained with *L. variegatus* embryos (not shown). During later development, the cytoplasmic fluorescence decreases while the nuclear fluorescence increases. By gastrula stage the majority of fluorescence is localized in or juxtaposed to the nuclei. Bar, 10 μ m.

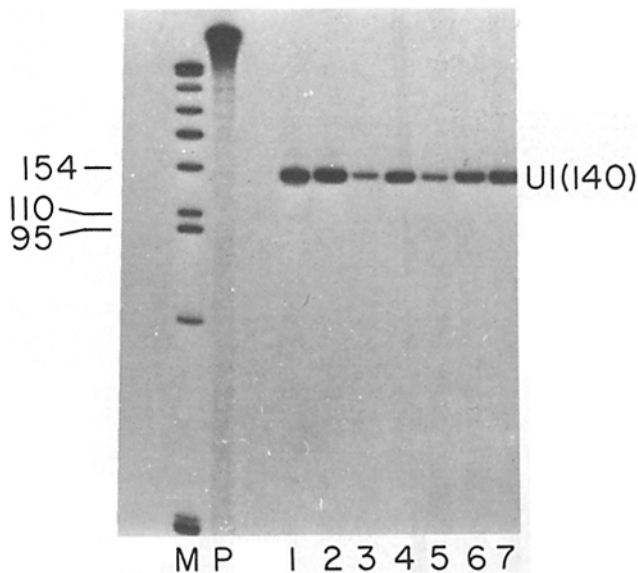


Figure 6. Analysis of UI RNA in nuclear and cytoplasmic fractions by S1 protection. RNA was prepared from *L. variegatus* eggs and from cytoplasmic and nuclear fractions of morula (64–128 cells), blastula (before hatching), and gastrula stage embryos. Equal cell equivalents of cytoplasmic and nuclear RNAs were analyzed for each stage (using 100 ng of cytoplasmic or total egg RNA). The RNA was hybridized with the sea urchin UI DNA probe, LvUI.1, labeled at the Bgl II site at the 5' end, nucleotide 140 of the UI RNA. The S1-resistant DNA was analyzed by PAGE and visualized by autoradiography. (Lane 1) Egg RNA; (lanes 2 and 3) cytoplasmic and nuclear RNA, respectively, from morula; (lanes 4 and 5) cytoplasmic and nuclear RNA from blastula; (lanes 6 and 7), cytoplasmic and nuclear RNA from gastrula. *M* is pUC18 DNA digested with Hpa II and *P* is the parental DNA fragment.

nuclear, as illustrated in Fig. 2, *d-f*, which shows sections of an early blastula (12 h, or ~170 cells), a gastrula (42 h), and a pluteus larva (68 h), respectively. At the latter two stages we used sections 1- μ m thick to obtain better resolution and to avoid quenching of nuclear signals. At these stages all nuclei are labeled to approximately uniform intensity and little signal is detectable in the cytoplasm.

Localization of Anti-RNP Antigen

The snRNAs are found in ribonucleoprotein particles (snRNPs), each class of which contains a common set of proteins (Lerner and Steitz, 1979; Hinterberger et al., 1983) recognized by sera of the Sm specificity from some patients with lupus erythematosus. Other sera, anti-RNP, are specific for determinants found only on UI RNP (White and Hoch, 1981; Petterson et al., 1984). Sm and RNP antigenic determinants have been highly conserved during evolution and the human sera react efficiently with sea urchin snRNPs (Brown et al., 1985).

Using anti-RNP serum we localized UI snRNP proteins by indirect immunofluorescence microscopy as shown in Figs. 4 and 5. In most respects, the distributions observed were very similar to those found for UI RNA, and essentially identical results were obtained using anti-Sm antibodies (data not shown). Fig. 4 *A* shows that in large oocytes RNP antigen is present at highest concentration in germinal vesi-

cles, although significant fluorescence is also observed over the cytoplasm of some oocytes. In unfertilized mature eggs (Fig. 4 *C*) fluorescence is primarily cytoplasmic, with little signal detectable over pronuclei. After fertilization, the RNP antigen remains cytoplasmic through pronuclear fusion (Fig. 4 *E*), and 4-cell (Fig. 4 *G*) and 16-cell (Fig. 5 *A*) stages. The only difference that we detected in the general distribution of RNP antigen and UI RNA is that the antigen does not accumulate in micromere nuclei. Starting at early blastula stage, the RNP signal shifts progressively from cytoplasm to nuclei. In early blastulae (Fig. 5 *C*) fluorescence begins to concentrate in and around the nuclear regions of all cells, except for those in mitosis, in which it is more uniformly distributed. By hatching blastula (Fig. 4 *E*) signals are more restricted to nuclei, and by gastrula (Fig. 4 *G*) the antigen is localized predominantly within nuclei.

Biochemical Analysis of UI RNA Content of Nuclei and Cytoplasm

We used an S1 nuclease protection assay to determine the relative amounts of UI RNA in nuclear and cytoplasmic fractions prepared from later stages of *L. variegatus* embryos. The data in Fig. 6 show that the great majority of UI RNA is in the cytoplasmic fraction of morulae (about 70-cell stage), and >50% is still cytoplasmic at blastula stage. By gastrula the majority of UI RNA is nuclear. In these experiments we used a very gentle homogenization procedure to avoid nuclear lysis, and the results may overestimate nuclear content of UI RNA at earlier stages (where in situ hybridization data imply that most of this RNA is cytoplasmic), due to contamination of nuclear fractions by small amounts of cytoplasm. Assays of RNA from highly purified nuclei prepared by the method of Morris and Marzluff (1985) show that nuclei of embryos at midcleavage (about 128 cells) do contain UI RNA (data not shown).

At Least Some Maternal Cytoplasmic UI RNA Is Contained in Ribonucleoprotein Particles

The simplest interpretation of the fact that UI RNA as well

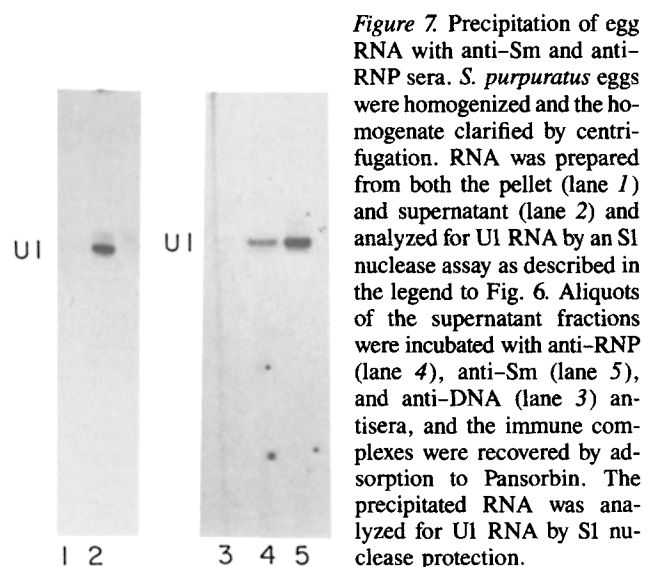


Figure 7. Precipitation of egg RNA with anti-Sm and anti-RNP sera. *S. purpuratus* eggs were homogenized and the homogenate clarified by centrifugation. RNA was prepared from both the pellet (lane 1) and supernatant (lane 2) and analyzed for UI RNA by an S1 nuclease assay as described in the legend to Fig. 6. Aliquots of the supernatant fractions were incubated with anti-RNP (lane 4), anti-Sm (lane 5), and anti-DNA (lane 3) antisera, and the immune complexes were recovered by adsorption to Pansorbin. The precipitated RNA was analyzed for UI RNA by S1 nuclease protection.

as RNP and Sm antigens are found together in the cytoplasm is that at least some of them exist as RNPs. We tested this hypothesis by determining whether the cytoplasmic U1 RNA of the egg could be precipitated by anti-RNP and anti-Sm antisera. Fig. 7 shows that both antisera precipitate egg U1 RNA. We have previously shown that both antisera also precipitate nuclear U1 RNA (Brown et al., 1985). These data suggest that egg U1 RNA is contained in an RNP particle that is similar to that of nuclear U1 RNP. In particular, the egg U1 RNP includes the anti-RNP determinant specific for U1 RNPs as well as at least some of the common snRNP proteins. However, because this analysis was not quantitative, we cannot conclude that all the U1 RNA is contained in particles precipitable by both antisera. It is also possible that the egg contains an excess of free snRNP proteins.

Nuclear Restriction of Newly Synthesized U1 RNA

Both U1 RNA and snRNP proteins are stored in the egg cytoplasm, and at least some of these molecules are assembled in RNP structures. However, the failure of these molecules to accumulate in the nuclei of eggs or early cleavage stage embryos, and the fact that accumulation of both proteins and RNA in nuclei is detectable only after new embryonic synthesis of U1 RNA begins (first detectable at 32- to 64-cell stage; Nijhawan and Marzluff, 1979), suggest that maternal snRNA(P)s are different from those newly synthesized. We therefore compared the distributions of total and of newly synthesized U1 RNA between nuclear and cytoplasmic fractions. Embryos of *S. purpuratus* were labeled with $^{32}\text{PO}_4$ from 16-cell to mesenchyme blastula stage, and RNA was extracted from nuclear and cytoplasmic fractions and analyzed by gel electrophoresis. The total U1 RNA content of nuclear and cytoplasmic fractions was compared using embryos from a parallel culture. Fig. 8 shows that a large fraction of bulk U1 RNA is cytoplasmic in *S. purpuratus* blastulae, as was demonstrated above for *L. variegatus*. In contrast most, if not all, of the U1 RNA synthesized between 16-cell and blastula stages is confined to embryo nuclei. These newly synthesized molecules must represent a significant fraction of total U1 RNA at blastula stage because the steady-state concentration of U1 RNA increases more than threefold by this stage (Brown et al., 1985). We conclude that newly synthesized and maternal U1 RNAs do not equilibrate during this period.

Discussion

Our data suggest that maternal and embryonic U1 RNAs are segregated during postfertilization development. New transcripts are confined to nuclei while most, if not all, maternal molecules are cytoplasmic. While we cannot exclude the possibility that some maternal U1 RNP re-enters embryonic nuclei, such a partial equilibration of maternal and embryonic U1 RNA transcripts seems unlikely. This segregation is particularly intriguing because U1 RNPs are assembled in the cytoplasm (Elicieri, 1980; Madore et al., 1984; Fisher et al., 1985) and during the frequent nuclear divisions of cleavage new U1 RNAs and RNPs at least transiently cohabit the same cytoplasm with maternal molecules. While it is possible that maternal U1 snRNPs are sequestered in some unknown manner on release from the germinal vesicle at

maturation, it seems more likely that there is some structural difference between maternal and embryonic snRNAs or snRNPs. This could be mediated by differences in primary sequence, but the predominant maternal and late embryonic U1 gene repeats of *S. purpuratus* differ by only one nucleotide (Nash, M. A., and W. F. Marzluff, unpublished results). Furthermore, in another sea urchin species, *Lytechinus variegatus*, maternal and embryonic U1 RNAs are identical (Yu et al., 1986). The more likely alternative is that the maternal snRNPs may be subjected to some alteration after maturation, possibly including protein modification and/or changes in protein composition. Although we do not yet know whether the cytoplasmic maternal snRNPs are ever functional after oocyte maturation, it is worth noting that both *Xenopus* and sea urchin oocytes and eggs contain large amounts of cytoplasmic polyadenylated RNA that resemble partially processed nuclear transcripts (Costantini et al., 1980; Anderson et al., 1982). While no postfertilization processing of these transcripts has been demonstrated, R. Ruzdijic and T. Pederson (personal communication) have recently shown that at least some cytoplasmic U1 RNP is associated with them. Alternatively, maternal U1 snRNPs may simply represent part of the residue of oogenesis, and be slowly turned over and diluted out.

The concentration and location of snRNAs and snRNPs have previously been studied during oogenesis and early development of *Xenopus laevis*. U1 snRNPs are very abundant

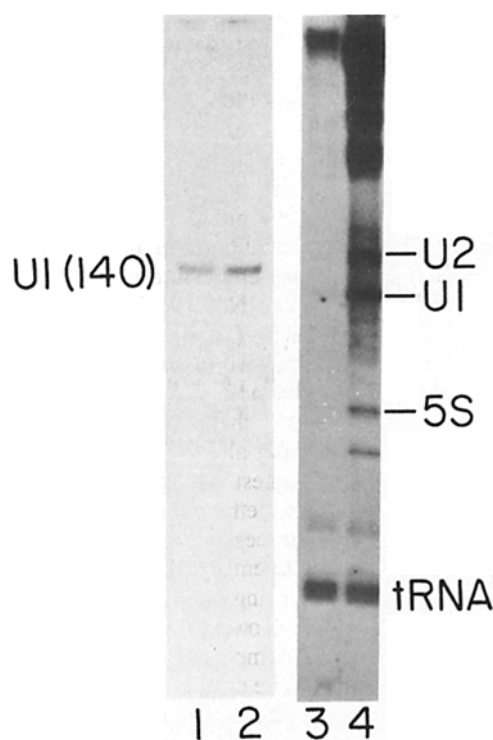


Figure 8. Newly synthesized U1 RNA is present in embryo nuclei. *S. purpuratus* embryos were labeled with $^{32}\text{PO}_4$ from 16-cell stage until after hatching. Equal cell equivalents of nuclear and cytoplasmic RNAs were analyzed by gel electrophoresis. Nuclear and cytoplasmic RNAs from a parallel culture were also analyzed by the S1 nuclease assay. Cytoplasmic RNA (lane 1) and nuclear RNA (lane 2) analyzed by the S1 nuclease assay; cytoplasmic RNA (lane 3) and nuclear RNA (lane 4) labeled with $^{32}\text{PO}_4$.

in germinal vesicles of previtellogenic oocytes, and U1 RNA amounts probably remain constant during vitellogenesis (Fritz et al., 1984). In contrast to sea urchin oocytes, the concentration of snRNP proteins is higher in cytoplasm of full-grown *Xenopus* oocytes than in germinal vesicles (Zeller et al., 1983; Fritz et al., 1984). Much of this protein is not complexed with U1 RNA but appears to consist of functional proteins because they can assemble microinjected snRNAs into snRNPs and translocate them to the nucleus (DeRobertis et al., 1982; DeRobertis, 1983). These observations have suggested a general mechanism in which nuclear accumulation is a property of the U1 snRNPs per se, and not an independent property of either the RNA or the proteins (Zeller et al., 1983). It is therefore surprising to find the Sm and RNP antigens and large quantities of U1 RNA in the cytoplasm of unfertilized sea urchin eggs and early cleavage stage embryos, and this observation further supports the idea that some component required for nuclear accumulation is absent or altered.

The metabolism of U1 snRNPs relates in interesting ways to what is known about the synthesis and processing of primary transcripts during oogenesis and early embryogenesis in sea urchins. The high concentration of U1 snRNP in oocyte nuclei is consistent with its role in processing of functional maternal mRNAs synthesized during oogenesis. Although sea urchin eggs retain about the same number of molecules of U1 RNA as are present in oocytes (10⁷; Brown et al., 1985), (a) the fact that the grain density observed over egg nuclei after in situ hybridization is 10- to 20-fold lower than that over cytoplasm, and (b) the ratio of nuclear to cytoplasmic volumes (about 1:300 in fixed tissue; DeLeon et al., 1983), both suggest that fewer than 3,000 maternal molecules reaccumulate in the haploid pronuclei of mature eggs or in embryonic nuclei during early cleavage stages. In contrast, the U1 RNA content during the blastula-pluteus period is 30,000–75,000 molecules per nucleus. Although this might reflect a very low rate of nuclear RNA synthesis in eggs, Brandhorst (1980) has shown that egg nuclei synthesize RNA at rates several-fold higher than do nuclei of late stage embryos. The paucity of U1 RNA correlates with the low poly A content of egg pronuclei (Angerer and Angerer, 1981). They also accumulate massive amounts of histone mRNAs, which must have a lifetime in the nucleus that is several orders of magnitude longer than that of typical nuclear RNA populations (DeLeon et al., 1983; Showman et al., 1983). These observations suggest that egg pronuclei are transcriptionally active, but quite inefficient in at least some aspects of RNA transport and processing.

Unlike *Xenopus* and *Drosophila* embryos, sea urchin embryos synthesize nuclear RNA during early cleavage stages (for review see Davidson, 1976). However, we do not know when a typical spectrum of high complexity nuclear RNA is first synthesized and processed. The only identified mRNAs made during early cleavage are the early histone mRNAs, which account for ~30% of total mRNA synthesis in the 64- to 128-cell embryo (Maxson and Wilt, 1981) and are neither polyadenylated nor spliced (for review see Hentschel and Birnstiel, 1980). Solution hybridization measurements first detect a high complexity nuclear RNA population at 16-cell stage (Ernst et al., 1980), and our results suggest that some cells may become competent to process transcripts about this time. In a previous study we first detected new synthesis of

U1 RNA after 32-cell stage (Nijhawan and Marzluff, 1979). The in situ analysis presented here demonstrates U1 RNA ~1 h earlier in nuclei of micromeres of the 16-cell embryo. This may represent small amounts of new synthesis undetectable in our previous study, and these U1 RNA molecules may hybridize in situ with higher efficiency if they are not yet complexed with proteins, as suggested by the lack of corresponding accumulation of RNP and Sm antigens in these nuclei. The nuclear signals alternatively could result from reaccumulation of maternal U1 RNA molecules. Previous studies have also shown that micromeres inherit a distinct domain of egg cytoplasm (reviewed by Angerer and Davidson, 1984). In particular, micromeres lack ~25% of maternal RNA sequence complexity found in other blastomeres (Rodgers and Gross, 1978), and these sequences do not appear to be associated with functional polysomal mRNAs (Ernst et al., 1980). Micromeres are the first cells of the embryo whose fate is determined (Okazaki, 1975; Harkey and Whiteley, 1980), and the observation that their nuclei contain U1 RNA suggests that they also may be the first blastomeres to initiate normal RNA processing.

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