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Evidence for an association between U1 RNA and interspersed repeat single-copy RNAs in the cytoplasm of sea urchin eggs†

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† We dedicate this paper to the memory of Alberto Monroy (1913–1986)

Summary

Psoralen crosslinking of RNA–RNA intermolecular duplexes in sea urchin egg extracts reveals that some maternal poly(A)$^+$ RNA molecules are complexed with U1 RNA, a cofactor in somatic nuclear pre-mRNA splicing. Reaction of egg extracts with a monoclonal antibody specific for U1 snRNP selects, in addition to U1, RNAs that contain repeated sequences interspersed with single-copy elements. Antibody-selection experiments with nucleate and anucleate egg halves demonstrate that most of the U1 RNA–interspersed RNA complexes are cytoplasmic, as is the egg's store of total U1 snRNP. These results raise the possibility that maternal interspersed RNAs include unprocessed pre-messenger RNA molecules in arrested complexes with splicing cofactors.

Key words: oogenesis, maternal RNA, mRNA processing, sea urchin, egg, single-copy RNA.

Introduction

Sea urchin eggs incubated with sufficient actinomycin to block most transcription display a postfertilization increase in protein synthesis similar to that of untreated embryos (Gross, Malkin & Moyer, 1964; Gross & Cousineau, 1964). A similar result is obtained with anucleate halves of fertilized eggs (Tyler, 1963). These experiments established that the templates for protein synthesis in the early embryo are largely maternal and that the immediacy with which the zygotic genome takes control of early echinoderm development is less than had been previously thought (see Gross, 1964, for a lucid discussion).

Later, in the course of investigations on the sequence complexity of sea urchin egg RNA (Constantini, Britten & Davidson, 1980), a population of poly(A)$^+$ RNA was encountered with a property unexpected of mRNA, namely the presence of multiple repetitive sequences linked to single-copy DNA transcripts. These unanticipated interspersed egg RNAs were discovered by virtue of the fact that both strands of each transcribed repeated DNA sequence family are represented in different RNAs, so that these molecules form intermolecular duplexes when annealed (Constantini *et al.* 1980).

A comparable population of poly(A)$^+$ interspersed RNAs has been described in *Xenopus* oocytes (Anderson *et al.* 1982). In both phyla, this maternal RNA population represents a substantial fraction (50–70 %) of the total poly(A)$^+$ RNA. Interspersed RNA has a cytoplasmic localization in *Xenopus* oocytes (Anderson *et al.* 1982) and, given the small size of the sea urchin egg pronucleus, it is probable that the massive interspersed RNA population also has a cytoplasmic localization in this species (which is directly demonstrated in this paper).

Surprisingly, the interspersed RNA of *Xenopus* oocytes is not translatable in either wheat germ or reticulocyte lysates, or upon microinjection into cognate oocytes (Richter, Smith, Anderson & Davidson, 1984). What then is its functional significance, if any? In at least four respects, oocyte and egg interspersed RNA resembles the nuclear pre-mRNA (hnRNA) of somatic cells. (1) It is larger than embryo polyribosomal messenger RNA (Sconzo *et al.* 1974; Posakony, Flytzanis, Britten & Davidson, 1983). (2) It contains covalently linked repetitive and single-copy DNA sequence transcripts, as does echinoderm and mammalian hnRNA (Smith, Britten & Davidson, 1974;

Darnell & Balint, 1970; Jelinek *et al.* 1974; Holmes & Bonner, 1974). (3) Its repetitive sequences form intermolecular duplexes upon annealing (compare Constantini *et al.* 1980 with Federoff, Wellauer & Wall, 1977). (4) Interspersed RNA contains sequences homologous to *bona fide* polyribosomal mRNAs (fig. 6 in Constantini *et al.* 1980). Hence the available data indicate that interspersed repeat RNAs contain mRNA-homologous sequences embedded in larger, more structurally complex molecules. These considerations have led to speculation that interspersed RNAs might represent a store of incompletely processed mRNA precursor molecules (Davidson & Posakony, 1982; Davidson, Hough-Evans & Britten, 1982; Thomas *et al.* 1981; Thomas, Britten & Davidson, 1982; Anderson *et al.* 1982).

We have investigated this hypothesis with respect to the possibility that sea urchin egg interspersed RNA might be stably associated with a known cofactor in mRNA processing, U1 small nuclear RNA. Our reasons for considering, and then pursuing, this idea were as follows. First, the free energy change that takes place upon base pairing of the 5′ end of U1 RNA with donor splice sites in pre-mRNA is sufficiently large ($\Delta G°$ = approx. -15 kcal mol^{-1} (1 cal$_{th}$ = 4·184 J)) that one would expect such duplexes to be quite stable in the absence of splicing (see, for example, Mount *et al.* 1983). Second, unspliced mammalian pre-mRNA can be exported to the cytoplasm, showing that such molecules are not obligatorily nucleus-restricted (Carlock & Jones, 1981). Finally, we reasoned that if there were an accumulation of stable U1 RNA–pre-mRNA complexes in the cytoplasm of sea urchin eggs, their detection should be possible by techniques that have previously revealed the more short-lived (and lower-abundance) U1 RNA–pre-mRNA complexes that form transiently during mRNA processing in somatic nuclei (Calvet & Pederson, 1981; Setyono & Pederson, 1984). This has proven to be the case.

Materials and methods

Gametes, fertilization and fractionation of eggs and embryos

The experiments reported in this paper were done with both *Strongylocentrotus purpuratus* and *Lytechinus pictus*, which were maintained in the marine animal facility of this institution. The data reported were obtained with both species unless noted. 'Dry' sperm was picked up on a toothpick from excised testes. Eggs were shed from females injected intracoelomically with 0·55 M-KCl into Millipore-filtered sea water (obtained at the Marine Biological Laboratory, Woods Hole, MA). For embryo experiments, eggs were fertilized and embryos reared in filtered sea water (containing penicillin at 20 μg ml^{-1}) at 15°C with constant slow stirring.

Eggs (or embryos) were resuspended in 10 mM-NaCl, 1·5 mM-$MgCl_2$, 10 mM-Tris–HCl, pH 7·2 containing 10 units ml^{-1} of RNasin (Promega Biotech, Madison, WI, USA) and 5 mM-mercaptoethanol. For psoralen crosslinking experiments, 4′-aminomethyl-4,5′,8-trimethylpsoralen (AMT, HRI Associates, Emeryville, CA) was added to a final concentration of 1 mg ml^{-1}. The suspension was then sonicated for 5–10 s (Branson model W-375, standard microtip probe, power setting of 4–5). The sonicate was then transferred to appropriately sized polystyrene sulphonate tissue culture flasks and irradiated at 365 nm for 30 min at 4°C; flux = approx. 6000 μW cm^{-2} (see Calvet & Pederson, 1981).

After 365 nm irradiation to photocatalyse psoralen RNA–RNA crosslinks, the egg sonicates were mixed with 1 vol. of a solution containing 10 M-urea, 0·1 M-sodium acetate, pH 4·0, 20 mM-EDTA, 10 mM-EGTA, 1 % sodium dodecyl sulphate, 10 mM-2-mercaptoethanol. Solid CsCl was then added to 1 g ml^{-1}. When dissolved, the solution was layered over a cushion of 5·7 M-CsCl (in 10 mM-EDTA) in Beckman Quick-Seal tubes and centrifuged at 39 000 revs min^{-1} for 20 h in a Beckman 60Ti rotor. The pelleted RNA was dissolved in 0·125 M-NaCl, 10 mM-Tris–HCl, pH 7·5, 1 % sodium dodecyl sulphate, 1 mM-EDTA and precipitated twice in 65 % ethanol at −20°C.

For oligo(dT)-cellulose chromatography, the RNA was dissolved in water and heated at 65°C for 5 min, cooled to room temperature and made 0·4 M in NaCl and 10 mM in Tris–HCl, pH 7·2. The RNA was applied at room temperature to a column of oligo(dT)-cellulose. After washing the column with binding buffer, the bound poly(A)$^+$ was eluted with 10 mM-Tris–HCl, pH 7·2, made 0·1 M in sodium acetate and precipitated with 65 % ethanol at −20°C.

The poly(A)$^+$ RNA was dissolved in water and a portion was irradiated at 254 nm to cleave the psoralen crosslinks (see Calvet & Pederson, 1981, for details). RNA samples were then electrophoresed in 10 % polyacrylamide gels containing 7 M-urea, 1·25 mM-EDTA and 45 mM-Tris–borate, pH 8·3. RNA gel blot hybridizations were done by electrotransfer to diazobenzyloxymethyl paper or Gene Screen Plus (New England Nuclear Corp.), followed by hybridization with a sea urchin U1 DNA probe (Brown *et al.* 1985) labelled with ^{32}P-dATP by nick-translation. Hybridization was carried out at 42°C in 0·6 M- or 0·75 M-NaCl, 0·06 M- or 0·075 M-sodium citrate, 50 % formamide, 0·05 % each of bovine serum albumin, Ficoll and polyvinylpyrrolidone, 1 % SDS, 1 mM-EDTA, salmon sperm DNA (100–200 μg ml^{-1}) and 1·0–1·2×10^6 disints min^{-1} of the U1 DNA probe per ml. Specific activity of the probe ranged from 1 to 3×10^8 disints μg^{-1}.

Antibody selection experiments

Eggs (or egg halves, see below) were resuspended in 0·15 M-NaCl, 1·5 mM-$MgCl_2$, 10 mM-Tris–HCl, pH 8·5, containing RNasin at 5 units ml^{-1} and sonicated as described above. The sonicate was centrifuged for 10 min at 12 000 revs min^{-1} in a Sorvall HB-4 rotor and the supernatant was made 0·1 % (vol./vol.) in Nonidet P-40 detergent. 100 μg of RNP or Sm antibody (or nonimmune mouse IgG) were added

per ml of supernatant and the samples were incubated for 30 min on ice. Antigen–antibody complexes were isolated on protein A–Sepharose beads as described previously (Wieben & Pederson, 1982; Economidis & Pederson, 1983).

Separation of nucleate and anucleate egg halves

Egg halves were prepared by a modification of the classic procedure (Harvey, 1936). Each millilitre of packed eggs (obtained by a 3–5 min centrifugation at 1000 *g*) was resuspended gently in 4 ml of filtered sea water and layered on 15 ml of 0·75 M-sucrose (in sea water) over 15 ml of 1·0 M-sucrose. The samples were centrifuged in a Beckman SW28 rotor at 6000 revs min^{-1} for 10 min at 4°C. (Nucleated halves band just beneath the sea water–0·75 M-sucrose interface and the anucleate halves band at the 0·75–1·0 M-sucrose interface.) The recovered egg fractions were diluted in 5–10 vol. of sea water, carefully examined by phase-contrast microscopy and collected by centrifugation at 2000 *g* for 3 min. Preparation of extracts of egg halves for antibody selection experiments was as described in the preceding section for intact eggs.

Slot hybridization of antibody-selected RNAs

RNA eluted from antibody protein-A Sepharose beads was recovered by phenol/chloroform extraction and ethanol precipitation. The RNA was dissolved in 3 % formaldehyde, 1·5 M-NaCl, 0·15 M-sodium citrate, heat-denatured (65 %, 15 min), cooled and applied to nitrocellulose sheets using a 'Minifold II' slot apparatus (Schleicher and Schell, Inc., Keene, NH, USA). Hybridization was carried out as detailed for gel blots in the above section. The *S. purpuratus* maternal interspersed clones pSp53 (repeat) and pSp10HB (single-copy) (derived from the parental cDNA clone 2109A-10 of Posakony *et al.* 1983) and the clone pZCyI, corresponding to the 3′ untranslated region of an egg non-muscle actin mRNA (Lee *et al.* 1984), were kindly provided by Frank Calzone and Eric Davidson (California Institute of Technology) and labelled by nick-translation.

Results

Strategy

Two complementary experimental approaches were used in this study (Fig. 1). In one, homogenates of eggs or embryos were incubated with the psoralen derivative aminomethyltrioxsalen (AMT), a photochemical crosslinking reagent for base-paired regions of nucleic acids (Calvet & Pederson, 1979, 1981; Cimino, Gamper, Issacs & Hearst, 1985). Poly(A)$^+$ RNA was isolated under denaturing conditions (left branch, Fig. 1) and the presence of crosslinked U1 RNA was analysed by gel blot hybridization with a sea urchin U1 DNA probe. This procedure was used previously to detect the base pairing of U1 and U2

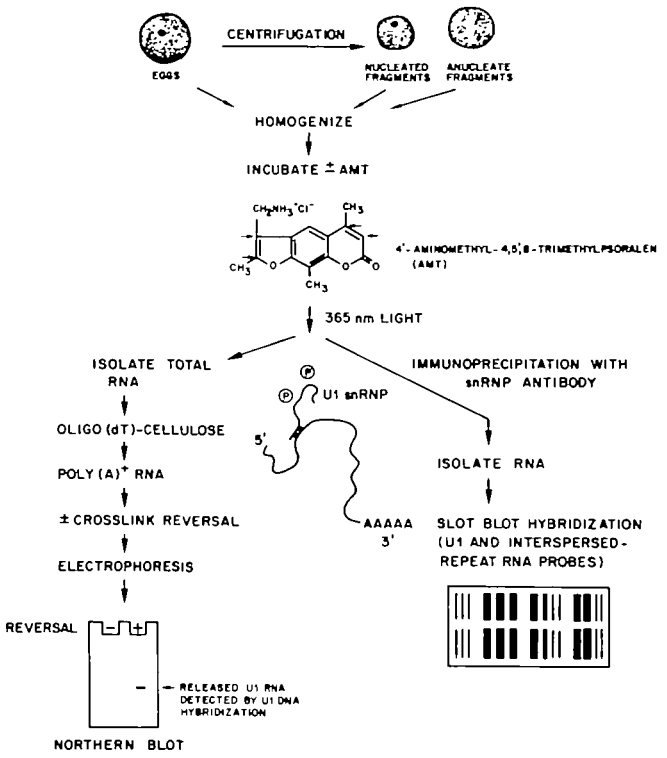

Fig. 1. Experimental design. After incubation of egg extracts with AMT and 365 nm irradiation, poly(A)$^+$ RNA was isolated and analysed for the presence of crosslinked U1 RNA (left branch of diagram). Alternatively, egg extracts were reacted with a U1 snRNP-specific antibody and the selected RNA was analysed by slot blot hybridization with probes for either U1 or interspersed RNAs (right branch of diagram). See Materials and Methods and Results sections for further details. The RNA duplex at the centre of the diagram illustrates schematically the U1 RNA–interspersed RNA complexes investigated in this study.

RNAs with mammalian nuclear pre-mRNA (Calvet & Pederson, 1981; Calvet, Meyer & Pederson, 1982).

The second experimental strategy (right branch, Fig. 1) involved immunoselection of egg RNP complexes using antibodies specific for proteins known to be complexed with U1 snRNP. This approach was suggested by the finding that such antibodies react with U1 snRNP complexed with mammalian pre-mRNA (Setyono & Pederson, 1984). In addition, when we began this work it was already clear that U1 snRNP proteins are conserved between insects and mammals (Lerner *et al.* 1980; Mount & Steitz, 1981; Wieben & Pederson, 1982; Sass & Pederson, 1984), making it extremely probable that echinoderm U1 snRNP would also react with these antibodies (subsequently confirmed by Brown *et al.* 1985; and the present results). Following antibody selection, the U1 and interspersed RNAs were detected by hybridization with appropriate probes.

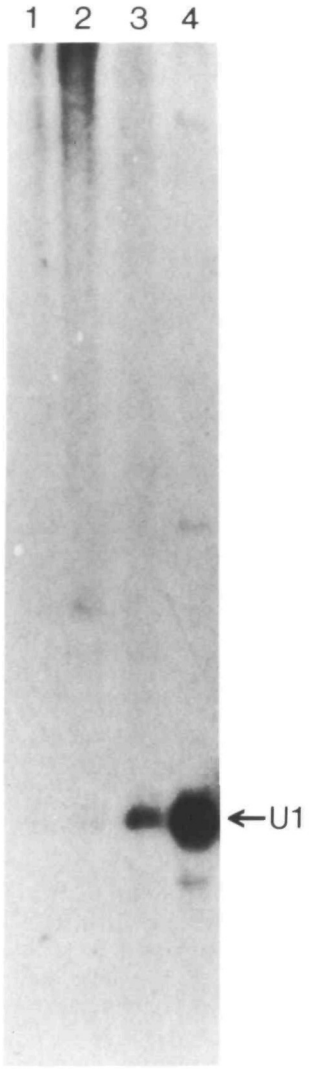

Fig. 2. AMT crosslinking of U1 RNA with egg poly(A)$^+$ RNA. Egg extracts were incubated with AMT, irradiated and poly(A)$^+$ RNA was analysed for crosslinked U1 RNA before or after reversal of the AMT crosslinks. Lane 1, no AMT; lane 2, AMT, crosslinks not reversed; lane 3, AMT, crosslinks reversed; lane 4, egg poly(A)$^-$ RNA for U1 RNA hybridization marker.

U1 RNA–poly(A)$^+$ RNA duplexes detected in egg and embryo extracts

Fig. 2 shows the typical results obtained when control or AMT-crosslinked poly(A)$^+$ RNA is analysed by electrophoresis and hybridization with a sea urchin U1 DNA probe. In the absence of crosslinking, there is no signal corresponding to U1 RNA (lane 1); this is expected since the non-adenylated U1 RNA (Brown *et al.* 1985) should not cofractionate with poly(A)$^+$ RNA on oligo(dT)-cellulose. In contrast, it can be seen in lane 3 that when AMT-crosslinked RNA is analysed after reversal of the crosslinks, a discrete RNA species appears which hybridizes with the U1 DNA probe and has the electrophoretic mobility

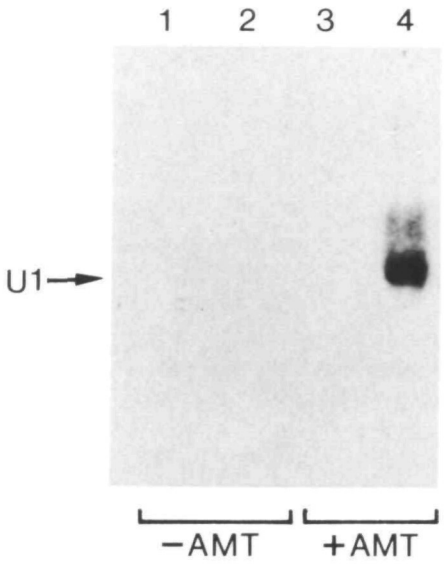

Fig. 3. Controls for AMT crosslinking specificity. Poly(A)$^+$ was isolated from untreated or AMT-treated egg extracts and analysed before or after crosslink reversal as in Fig. 2. Lane 1, no AMT, no reversal; lane 2, no AMT, mock-reversal; lane 3, AMT, no reversal; lane 4, AMT, reversal.

expected for U1 RNA (compare with U1 RNA standard in lane 4). A further, essential control in support of this conclusion is shown in Fig. 3. It can be seen in lane 2 that no U1 RNA signal appears when non-crosslinked RNA is mock-irradiated at 254 nm (the procedure used to cleave photochemically the AMT crosslinks); this shows that the U1 RNA observed in lane 3 of Fig. 2 (and in lane 4 of Fig. 3) is not generated by light-induced breakage of larger RNAs. U1 RNA–poly(A)$^+$ RNA complexes can be detected throughout early development (Fig. 4).

U1 snRNP antibody selects interspersed RNAs from egg extracts

From the foregoing results, it could be estimated that the amount of crosslinked U1 RNA was substoichiometric with respect to the poly(A)$^+$ RNA recovered from oligo(dT)-cellulose. We do not know if this is due to an inefficiency of the AMT crosslinking or, alternatively, to the association of U1 RNA with only a subset of poly(A)$^+$ RNA. In either case, the properties of specifically the U1 RNA-associated poly(A)$^+$ RNA obviously cannot be determined using the oligo(dT)-bound fraction. To circumvent this, we investigated whether antibodies against U1 snRNP can specifically immunoselect interspersed RNAs from egg extracts.

Mammalian U1 RNA is presently known to be complexed with ten proteins (Bringmann & Luhrmann, 1986). We have used two monoclonal antibodies that react with U1 snRNP. One, termed RNP,

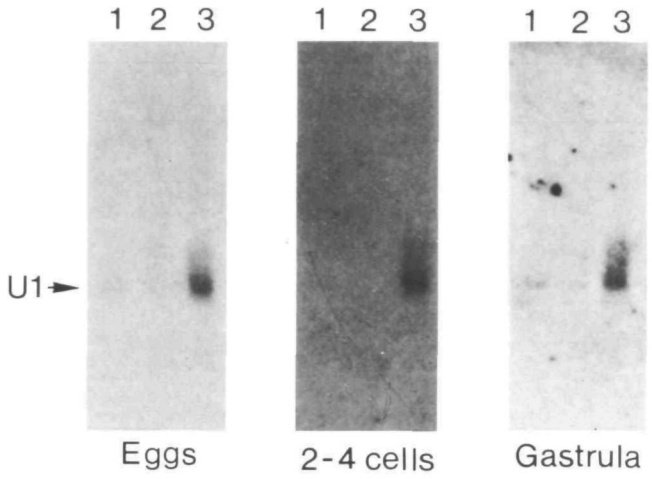

Fig. 4. U1 RNA-poly(A)$^+$ RNA crosslinking during early development. Extracts were prepared from eggs or embryos and analysed as in Figs 2, 3. Lane 1, no AMT; lane 2, AMT, no reversal; lane 3, AMT, reversal. This experiment was done on *Lytechinus pictus* material. Each lane contains between 8 and 10 μg of RNA. (While we have not systematically and quantitatively investigated early embryos, our results do show that there is no massive decrease in the level of U1 RNA–poly(A)$^+$ RNA duplexes scored by the AMT procedure from unfertilized eggs to gastrula.)

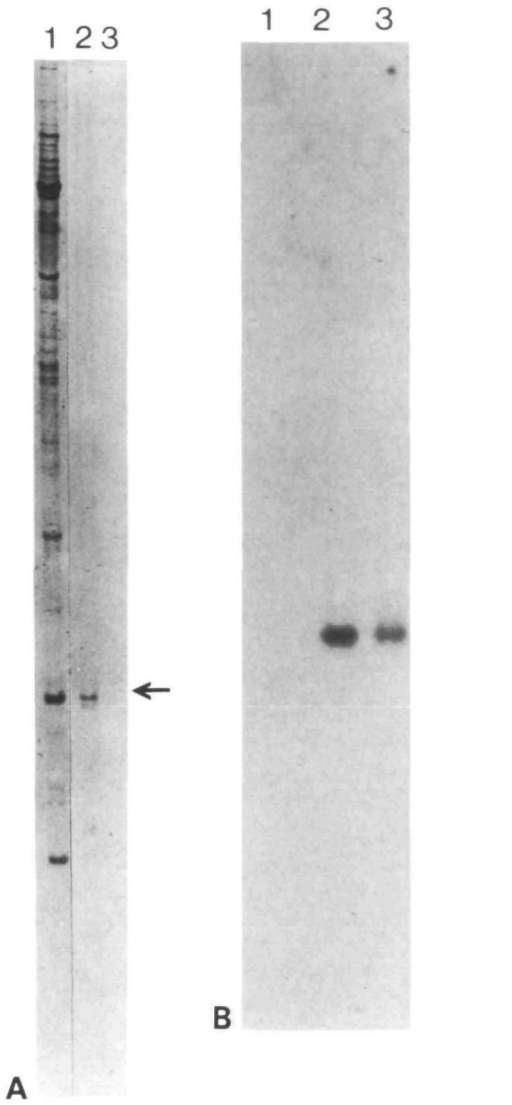

Fig. 5. Antibody specificity. (A) Ethidium-bromide-stained gel. Lane 1, egg poly(A)$^-$ RNA was fractionated on a sucrose gradient and the material sedimenting at between 5S and 15S was recovered. Lane 2, egg extract was reacted with RNP monoclonal antibody and the selected RNA was recovered on protein A–Sepharose (see Materials and Methods). Lane 3, same as lane 2 except that nonimmune IgG was used. Shown is a photograph of the negative of an ethidium-bromide-stained gel. The arrow indicates the position of U1 RNA as determined by gel blot hybridization conducted with parallel lanes. (The minor band migrating just below U1 RNA in lane 2 is a fragment of U1 that was not observed reproducibly.) (B) Gel blot hybridization of antibody-selected RNA with U1 DNA probe. Lane 1, nonimmune IgG; lane 2, RNP monoclonal antibody; lane 3, Sm monoclonal antibody.

reacts with an approximately 68 000 relative molecular weight protein of the U1 snRNP (Billings, Allen, Jensen & Hoch, 1982; Billings & Hoch, 1984). The second monoclonal antibody (Lerner, Lerner, Janeway & Steitz, 1981), termed Sm, reacts with three proteins that are part of the snRNPs that contain U1, U2, U4/U6 and U5 RNAs (Pettersson *et al.* 1984). The domains of snRNP proteins that react with these monoclonal antibodies and comparable human autoantibodies are conserved between vertebrates and arthropods (Lerner *et al.* 1980; Mount & Steitz, 1981; Wieben & Pederson, 1982; Sass & Pederson, 1984). It was therefore anticipated that these antibodies would also react with echinoderm U1 snRNP, as subsequently shown by Brown *et al.* (1985) and confirmed in Fig. 5. The ethidium bromide-stained gel in Fig. 5A shows that RNP antibody selects an RNA species (lane 2) that hybridizes with the sea urchin U1 probe (Fig. 5B, lane 2). The specificity of the antibody can be readily seen by comparison of lane 2 with the pattern of total RNA (Fig. 5A, lane 1). Sm monoclonal antibody reacts with U1 RNA and at least three additional RNAs (not shown). The presence of U1 RNA among the Sm-selected species is shown in Fig. 5B, lane 3.

RNP monoclonal antibody was next used to investigate the possible association of U1 snRNP with interspersed RNAs. The results of a typical experiment are shown in Fig. 6. It can be seen in the duplicate slots in column 2 that U1 snRNP antibody selects RNA molecules that hybridize with a probe for a characteristic repetitive sequence embedded in interspersed RNAs (compare signal with that

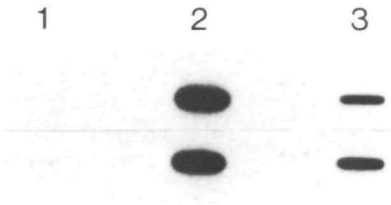

Fig. 6. RNP antibody reaction with interspersed RNAs. Egg extract was reacted with RNP monoclonal antibody and the selected RNAs were analysed by slot blot hybridization. Shown is a set of duplicate slots. Column 1, nonimmune IgG, interspersed repeat RNA probe; column 2, RNP antibody, interspersed repeat RNA probe; column 3, RNP antibody, interspersed single-copy probe.

obtained with non-immune IgG in column 1). In column 3, the same U1 snRNP antibody-selected egg RNA as shown in column 2 was hybridized with a probe corresponding to a single-copy sequence element present in interspersed RNAs. It can be seen that the antibody also selects these single-copy sequences.

Cytoplasmic localization of U1 snRNP–interspersed RNA complexes

To investigate the localization of egg U1 snRNP–interspersed RNA complexes, immunoselection experiments were undertaken using extracts from nucleate *versus* anucleate egg halves (see Materials and methods). To determine first the distribution of total U1 snRNP itself, extracts were reacted with antibody and the immunoselected RNA was hybridized with the U1 DNA probe. This revealed that U1 snRNP is distributed approximately uniformly between the nucleate and anucleate halves (Fig. 7). The nucleate egg halves prepared by this method are mostly cytoplasm; phase-contrast microscopy conducted in each experiment revealed that the percentage of egg cytoplasm in the anucleate and nucleate halves ranged from 40 and 60 %, respectively, to 60 and 40 %, respectively. (It was 50:50 % in the particular experiment shown in Fig. 7.) It follows that most of the U1 snRNP is present in the cytoplasm (see also Nash *et al.* 1987).

Immunoselection of nucleate and anucleate extracts followed by hybridization of the selected RNA with a probe for a repeat sequence characteristic of interspersed RNA typically revealed patterns such as shown in Fig. 8. In column A, it can be seen that RNP antibody selects interspersed RNAs from both the nucleate (slot 3) and anucleate fractions (slot 4). The ratio of cytoplasm in the two fractions from this particular experiment was approximately 35 % nucleate and 65 % anucleate (see drawings in column E). The results obtained with Sm antibody are shown

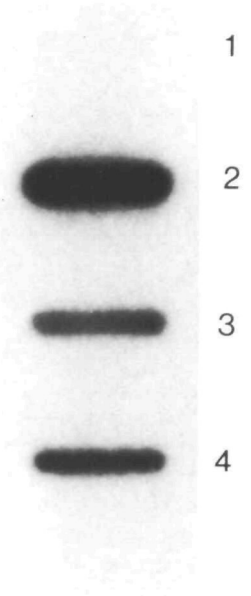

Fig. 7. Distribution of U1 snRNP between nucleate and anucleate egg halves. Extracts were reacted with Sm monoclonal antibody and the selected RNA was analysed by slot blot hybridization with the U1 DNA probe. Slot 1, whole egg, nonimmune IgG; slot 2, whole egg, Sm antibody; slot 3, nucleate halves, Sm antibody; slot 4, anucleate halves, Sm antibody. The amounts of RNA loaded on slots 3 and 4 was derived from the same number of eggs as used in slots 1 and 2. Results similar to those shown were obtained with RNP antibody.

in column B. With both antibodies, the amount of interspersed RNA selected from the nucleate plus anucleate halves adds up to that selected from an equivalent amount of total egg extract (in columns A and B, compare slot 2 with slots 3 plus 4). From these results, we conclude that the U1 snRNP–interspersed RNA complexes are localized mainly in the egg cytoplasm.

Over the course of several experiments, the amount of interspersed RNA immunoselected from nucleate *versus* anucleate extracts always paralleled the respective distribution of cytoplasm. From this observation, we infer that the U1 snRNP–interspersed RNA complexes detected by this hybridization probe are probably not associated with very large cytoplasmic structures (for example, yolk granules) that specifically partition into either egg half during the centrifugal force-imposed stratification.

We have also determined that total (as opposed to U1 snRNP antibody-selected) interspersed RNA reacting with this hybridization probe is equally distributed between nucleate and anucleate egg

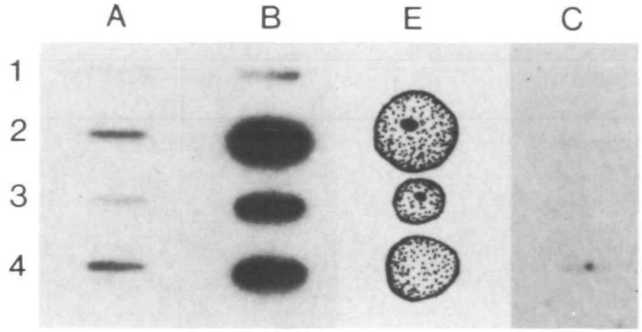

Fig. 8. Antibody reaction with interspersed RNA *versus bona fide* mRNA in nucleate and anucleate egg halves. Extracts from eggs or egg halves were reacted with RNP or Sm antibody and the selected RNAs were analysed by slot hybridization with probes for interspersed RNA or a *bona fide* mRNA. Column A, RNP antibody, interspersed RNA probe; column B, Sm antibody, interspersed RNA probe; column E ('eggs'), representative drawings made from phase-contrast microscopy; column C, RNP antibody, actin mRNA probe. Slot 1, whole egg, nonimmune IgG; slot 2, whole egg, RNP or Sm antibody; slot 3, nucleate halves, RNP or Sm antibody; slot 4, anucleate halves, RNP or Sm antibody. The particular RNP antibody used in this experiment was from a human autoimmune patient; it was as specific for both vertebrate and echinoderm U1 snRNP as the RNP monoclonal antibody. Results similar to those shown in column A were obtained with the RNP monoclonal. In the experiment shown, human nonimmune IgG was used as the control in slots 1 of columns A and C. The sea urchin portion of the actin mRNA hybridization probe used in column C had a length greater than that of the interspersed repetitive sequence probe; moreover, the two probes had virtually identical specific activities. Thus, the lack of actin mRNA signal cannot be attributed to a deficiency of the hybridization probe, as is also demonstrated by the strong signal obtained with the pZCyI probe and total egg poly(A)$^+$ RNA (data not shown).

halves (data not shown), showing that the interspersed maternal RNA population of sea urchin eggs is cytoplasmic.

To investigate the possible association of a *bona fide* messenger RNA with U1 snRNP, RNP antibody-selected RNA was hybridized with a probe for the 3′ untranslated region of a maternal actin mRNA. This mRNA is not detectably present in unprocessed form in the egg (F. Calzone & E. H. Davidson, personal communication of unpublished results). No reaction between antibody and actin mRNA could be detected in extracts of eggs, nucleate or anucleate halves under the experimental conditions employed (Fig. 8, column C). This negative control is particularly significant in that there is a similar number of copies of this actin mRNA per egg as there are of the interspersed repeat RNA sequence probed in columns A and B.

Discussion

Interspersed egg RNAs associated with U1 snRNP

We have performed two types of experiments to investigate the association of sea urchin egg U1 snRNP with poly(A)$^+$ and/or interspersed RNAs. It is, as always, necessary to consider these results in the context of the procedures used. Owing to the fact that psoralen is not taken up by sea urchin eggs (S. Ruzdijic & T. Pederson, unpublished data), it was not possible to conduct the crosslinking *in vivo* as we did previously in our experiments with mammalian cells (Calvet & Pederson, 1981; Calvet *et al.* 1982). We therefore cannot formally exclude the possibility that the U1 snRNP–interspersed RNA complexes observed in this study form during egg homogenization. Due to the specificity of psoralen for base-paired regions of RNA (Cimino *et al.* 1985), the crosslinking procedure would not score fortuitous RNA–RNA complexes unless an actual base-paired region were present. In addition, given the high dilution of intracellular RNA concentrations that attends preparation of the egg extracts and the low ionic strength (10 mM-Na$^+$) of the homogenization buffer, it is unlikely that bimolecular RNA–RNA duplex formation would occur during homogenization. We therefore favour the hypothesis that the U1 RNA–poly(A)$^+$ RNA duplexes detected in the psoralen crosslinking experiments exist in the living egg. The fact that a *bona fide* maternal messenger RNA is not immunoselected by RNP antibody (Fig. 8C) also suggests that the results reported here reflect a specific molecular interaction of U1 snRNP with some, but not all, maternal RNAs.

Only a small fraction of the egg's total content of U1 RNA is recovered in crosslinked complexes with poly(A)$^+$ RNA. This may reflect an inherent inefficiency of the procedure (an issue that complicates many crosslinking studies) or, alternatively, may signify that only a small subset of egg poly(A)$^+$ RNA is bound to U1 RNA. It is noteworthy that even in HeLa cell nuclear RNA, representing an active mRNA processing situation, only a small fraction of the U1 RNA is psoralen crosslinkable to pre-mRNA (Calvet & Pederson, 1981). In the same vein, we estimate that less than 10 % of the egg interspersed RNA is selected by U1 snRNP antibody in the immunoselection experiments. At present, we do not know whether this represents an inherent limitation of the antibody reaction (due, for example, to antigen masking on some U1 snRNP–interspersed RNA

complexes) or is a quantitative reflection of the true situation in the egg.

Cytoplasmic localization

We undertook these experiments to test the hypothesis that interspersed RNA in sea urchin eggs might be complexed with mRNA splicing machinery. The information available when we started indicated that egg interspersed RNA is cytoplasmic. We did not anticipate, however, that sea urchin egg U1 snRNP is itself localized in the cytoplasm (Fig. 7). While this paper was in preparation, we learned of *in situ* hybridization results demonstrating that the large majority (probably more than 99 %) of sea urchin egg U1 RNA resides in the cytoplasm (R. C. Angerer, personal communication of unpublished results; Nash *et al.* 1987).

The fact that the U1 snRNP–interspersed RNA complexes are localized in the cytoplasm of sea urchin eggs eliminates the possibility that they reflect ongoing nuclear pre-mRNA splicing. This was by no means a trivial possibility, since unfertilized sea urchin eggs do synthesize unstable heterogenous nuclear RNA (Levner, 1974; Dworkin & Infante, 1978) and, as pointed out above, our results reflect a relatively small fraction of the total egg interspersed RNA.

Possible origin and function of maternal interspersed RNA

How does cytoplasmic interspersed RNA arise during oogenesis? One possibility is that oocyte transcription is so intense that these RNAs 'slip through' the nuclear mRNA processing machinery. This hypothesis envisions that the amount of pre-mRNA transcribed exceeds the processing capacity of the oocyte nucleus. Interspersed RNAs might associate with snRNPs in the nucleus and yet fail to be acted upon by limiting processing activities. Alternatively, interspersed RNAs might combine with cytoplasmic stores of U1 and other snRNPs after nuclear export. We note that the presence of a cytoplasmic store of U1 snRNP in sea urchin eggs is not a condition required for the simultaneous presence of interspersed RNA. This emerges from the fact that, although *Xenopus* oocytes contain a comparable cytoplasmic interspersed RNA population, in this case U1 snRNP is restricted to the germinal vesicle (Fritz, Parisot, Newmeyer & De Robertis, 1984). This does not, of course, invalidate the hypothesis that interspersed RNA contains unprocessed mRNAs, but does indicate that the interaction with U1 snRNP reported here may be a particular feature of the *cytoplasmic* U1 snRNP storage strategy employed by echinoderms.

In the initial characterization of sea urchin egg interspersed RNAs, it was found that this RNA population contained virtually all of the single-copy sequences present in total egg RNA. Since the great majority of these same single-copy sequences are present in polyribosomes after fertilization (Constantini *et al.* 1980) (and are therefore *bona fide* mRNAs by this criterion), it follows that the egg interspersed repeat RNA fraction contains sequences homologous with exons. The hypothesis that the maternal interspersed RNA population contains unprocessed mRNAs also receives support from its larger than mRNA size (Sconzo *et al.* 1974; Posakony *et al.* 1983) and its content of repeated DNA sequence transcripts that are removed after fertilization (Duncan & Humphreys, 1981, 1983).

It is intriguing to consider that different pre-mRNAs might be tethered at fixed locations in the egg cytoplasm. Owing to such localization, nuclear envelope reformation following early cleavages might capture individual pre-mRNAs in certain nuclei but not others, wherein they might then undergo productive mRNA processing. To test this hypothesis, which is a variation on the classical theme of maternal (translatable) mRNA, it will be necessary to conduct further experiments examining the sequence structure and possible postfertilization translation of interspersed RNAs.

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