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Synthesis of U1 RNA in Isolated Nuclei from Sea Urchin Embryos: U1 RNA Is Initiated at the First Nucleotide of the RNA†

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Nuclei from sea urchin blastula embryos synthesize a variety of small RNAs, one of which has identical mobility with sea urchin U1 RNA. This RNA is synthesized by RNA polymerase II and, in a hybridization-selection experiment, was selected by the cloned sea urchin U1 gene. The U1 RNA was initiated with ATP, but not GTP, in isolated nuclei with β-*S*- and γ-*S*-ribonucleotide triphosphates as substrates. The U1 RNA containing thiophosphate at the 5′ end was not capped but accumulated as an uncapped transcript from which the thiophosphate could be removed with calf intestinal phosphatase.

One of the most abundant RNAs in most animal cells is the U1 snRNA (7). This RNA forms a characteristic ribonucleoprotein particle which can be immunoprecipitated with anti-RNP or anti-Sm antisera from patients with the autoimmune disease lupus erythematosus (18, 19). The sequence of this RNA has been highly conserved in evolution, with only a 25 to 30% difference between U1 RNA from such diverse organisms as rats, drosophila, and sea urchins (3, 5, 28). Much evidence is accumulating that U1 RNA functions in the biosynthesis of mRNA at the level of RNA splicing (17, 27, 31). However, little is known about the biosynthesis of U1 RNA.

Genes coding for U1 RNA have been isolated from a number of organisms. Although U1 RNA is transcribed by RNA polymerase II, genes from rats, humans, and mice are unusual in that they lack a TATAA sequence close to the 5′ end of the RNA (22, 23, 29, 35, 40). These genes have extensive homology in the 5′ flanking region extending 70 bases 5′ from base 1 of the RNA (23). The U1 genes also differ from other RNA polymerase II transcripts in that they show UV sensitivity very different from that predicted from the size of the RNA product (10, 12). Based on this UV sensitivity, it has been proposed that these RNAs are synthesized as part of a large transcript.

Larger U1 RNAs than mature U1 RNA have been detected in pulse-labeling studies in HeLa cells (11, 13, 21). These RNAs are present in the cytoplasm and have an extended 3′ end of 1 to 10 nucleotides (11, 21). It is not known whether this represents the primary transcript of the human U1 genes or whether this intermediate is formed by a processing reaction. These pre-U1 RNAs are found predominantly in the cytoplasm (13). They have already been assembled into ribonucleoprotein particles and are converted to mature U1 RNA during a chase (21).

The results with DNA-dependent transcription systems also demonstrate the difference in the biosynthesis of U1 RNA and the other RNA polymerase II transcripts. When a human U1 gene is used as a template in a HeLa cell-free system, RNA synthesis is initiated at nucleotide −183 rather than at nucleotide 1 of the RNA (29). The −183 initiation site is 30 to 40 bases downstream of the sequence TATGTAGA, which may function as a TATAA-like sequence in vitro. This TATAA-like sequence is required for transcription of the human U1 gene in *Xenopus* oocytes (35). In addition, a sequence centered about nucleotide −50 is required for correct positioning of the 5′ end. In the oocytes, transcription starts at nucleotide 1 of the RNA as judged by incorporation of [β-^{32}PO$_4$]ATP into the cap structure (35). Faithful synthesis of snRNAs has not been reported in a cell-free system, either in a DNA-dependent system or in one based on isolated nuclei.

We previously studied the gene organization and synthesis of sea urchin U1 RNA during embryogenesis. Sea urchin U1 RNA is encoded by a 1,400-base tandemly repeated gene set (8) and is synthesized at a high rate early in sea urchin development (30). Using isolated nuclei from sea urchin blastula embryos, we report that the U1 RNA is synthesized accurately in vitro. A large proportion of the U1 RNA is initiated in vitro based on the incorporation of adenosine 5′-*O*-(2-thiotriphosphate) (β-*S*-ATP) or adenosine 5′-*O*-(3-thiotriphosphate) (γ-*S*-ATP) into the U1 RNA. The U1 RNA is also terminated accurately. The U1 transcripts initiated with either β- or γ-*S*-ATP are not capped, suggesting that the thionucleotides inhibit the capping reaction.

MATERIALS AND METHODS

Isolation of nuclei and conditions of RNA synthesis. Nuclei were isolated exactly as previously described (26) from blastula embryos of either *Lytechinus variegatus* or *Strongylocentrotus purpuratus* just after hatching. Nuclei were stored in liquid N_2 at a concentration of 1 to 2 mg of DNA per ml (1 × 10^9 to 2 × 10^9 nuclei per ml) in 25% glycerol–5 mM $MgCl_2$–50 mM Tris hydrochloride (pH 8)–1 mM EGTA (ethylene glycol-bis[β-aminoethyl ether]-*N*,*N*,*N*′,*N*′-tetraacetic acid)–1 mM spermidine–0.1 mM phenylmethylsulfonyl fluoride–1 mM dithiothreitol. Nuclei were incubated with either [α-^{32}PO$_4$]GTP or [α-^{32}PO$_4$]CTP (25 to 50 μM) in 100-μl reactions exactly as previously described (26). In some experiments, 500 μM β-*S*-ATP, γ-*S*-ATP, or guanosine 5′-*O*-(3-thiotriphosphate) (γ-*S*-GTP) was substituted for the corresponding ribonucleotide. The RNA was prepared from both the nuclear and extranuclear fractions as previously described (26).

Chromatography on mercuriagarose. Unincorporated nucleotides were removed from the RNA by chromatography on Sephadex G-50. The RNA was recovered by ethanol precipitation. The RNA was applied to a 1-ml column of mercuriagarose (Affigel 501; Bio-Rad Laboratories, Rich-

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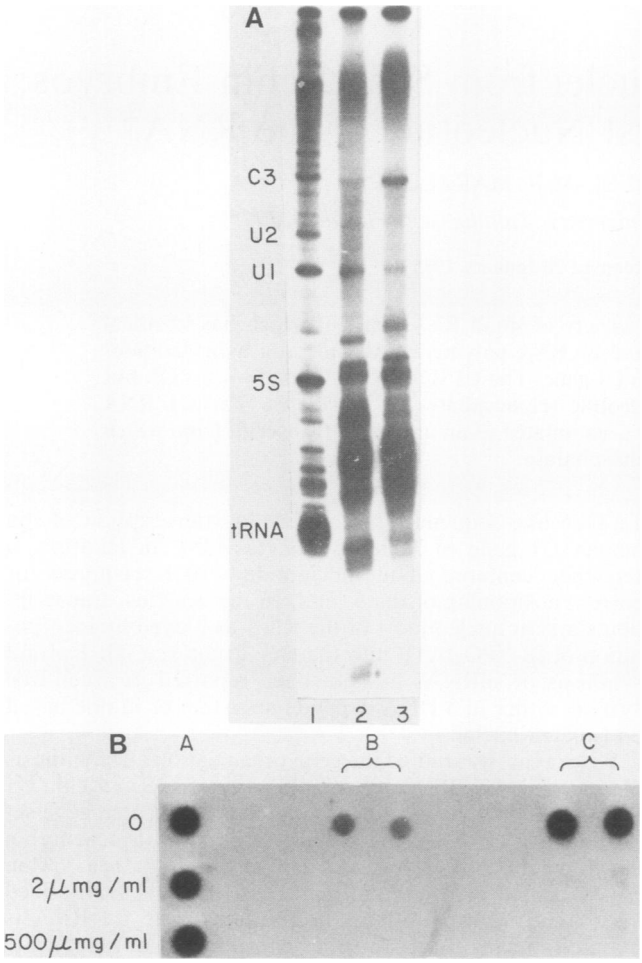

FIG. 1. Small RNAs synthesized in isolated nuclei. (A) Nuclei from either *S. purpuratus* (lane 2) or *L. variegatus* (lane 3) blastula embryos were incubated with [α-$^{32}PO_4$]GTP for 30 min. The RNA released from the nuclei was analyzed by gel electrophoresis. RNA prepared from *L. variegatus* embryos grown with $^{32}PO_4$ used as a marker (lane 1). C3, ●●●. (B) Nuclei from *L. variegatus* embryos were incubated with [α-$^{32}PO_4$]GTP with either 2 or 500 μg of α-amanitin per ml. The total RNA from the reaction was hybridized to cloned DNAs immobilized on nitrocellulose. The DNAs used were: A, rDNA, plasmid pLv4 (2); B, U1 DNA, plasmid pLvU1.1 (4); C, histone DNA, plasmid pSp102 (9).

mond, Calif.). Chromatography was performed exactly as described by Stallcup and Washington (38), except that all volumes were reduced by a factor of 3. The bound RNA was eluted from mercuriagarose with 10 mM dithiothreitol–0.1 M NaCl–0.1% sodium dodecyl sulfate (SDS)–1 mM EDTA–10 mM Tris hydrochloride(pH 8) as described by Stallcup and Washington (38). The RNAs in the bound and unbound fractions were recovered by ethanol precipitation and analyzed by either gel electrophoresis or RNA-DNA hybridization.

Gel electrophoresis. RNA was analyzed by electrophoresis on 10% polyacrylamide gels in 7 M urea as previously described (8). The RNA was detected by autoradiography.

RNA-DNA hybridization. Plasmid DNAs were denatured and immobilized on nitrocellulose with a Hybridot (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) apparatus as previously described (26). A total of 5 μg of DNA was applied per dot. The nitrocellulose strip was prehybridized for 2 h at 52°C in 2 ml of 50% (vol/vol) formamide–2 mM EDTA–0.75 M NaCl–0.075 M trisodium citrate–0.1% SDS–0.1% Ficoll–0.1% polyvinylpyrrolidone–25 μg of polyadenylic acid per ml–10 mM Tris hydrochloride (pH 7.5). The prehybridization solution was discarded, and the filter was hybridized in 0.2 to 0.3 ml of the same solution containing the radioactive RNA. The filters were washed three times (30 min each) with 50 ml of 0. 15 M NaCl–0.015 M trisodium citrate–0.1% SDS–2 mM EDTA–10 mM Tris hydrochloride (pH 7.5) at 52°C and rinsed for 2 min in 0.15 M NaCl–0.015 M trisodium citrate at room temperature before exposure to X-ray film. The dots were cut out with a punch and eluted with two successive 175-μl portions of 99% formamide–0.2% SDS–10 μg of yeast tRNA per ml at 52°C for 10 min. The solution was filtered through siliconized glass wool in a disposable pipette tip and recovered by ethanol precipitation. The RNA was then analyzed by gel electrophoresis.

S1 nuclease assay. The S1 assays with radioactive RNA were performed essentially as described previously (5). The RNA in the extranuclear fraction (prepared as described previously [26]) which was bound to mercuriagarose was hybridized to 1 μg of pLvU1.1 cut with either *Hin*dIII or *Bgl*II. S1 digestions (1,000 U/ml) were done at 37°C for 1 h without the addition of carrier DNA. The protected RNA was recovered by ethanol precipitation and redissolved in 80% formamide–5 mM Tris-borate (pH 8.3)–0.1 mM EDTA. The sample was heated at 65°C for 5 min to disrupt the RNA-DNA hybrids and was then analyzed by gel electrophoresis.

Phosphatase treatment of the RNA. The RNA was treated with 10 U of calf intestinal phosphatase (CIP) in 100 μl of 50 mM Tris hydrochloride (pH 8.0)–0.1 mM EDTA at 37°C for 1 h. The reaction was stopped by the addition of 100 μl of 0.2 M NaCl–2 mM EDTA–1% SDS and then by heating at 68°C for 15 min. The sample was extracted twice with an equal volume of water-saturated phenol-chloroform (2:1). The RNA was recovered from the aqueous phase by ethanol precipitation.

DNA probes. *L. variegatus* 5S DNA, pLu305, is a 1.0-kilobase fragment containing one copy of a 5S gene (20) cloned into pACYC184. *L. variegatus* ribosomal DNA, pLv4, is a 4.8-kilobase *Sal*I fragment containing the 3′ portion of the 26S rRNA and a portion of the nontranscribed spacer DNA (2). Both the above clones were provided by Darrell Stafford, University of North Carolina. *S. purpuratus* histone DNA, pSp102 (kindly provided by Larry Kedes, Stanford University) is a 4.6-kilobase fragment containing a single copy of the H1, H2b, and H4 early histone genes cloned in ColE1 (9). *L. variegatus* U1 DNA, pLvU1.1, is a 1.4-kilobase *Hin*dIII fragment inserted into the *Hin*dIII site of pBR322 (A). A subclone of pLvU1.1, pLvU1.1C, was used to generate the subclones in the bacteriophages m13mp8 and m13mp9. pLvU1.1C contains a 110-nucleotide *Sau*3A fragment extending from nucleotide 30 to nucleotide 140 of the U1 gene cloned into the *Bam*HI site of pUC9. The insert of pLvU1.1C was excised with *Eco*RI and *Hin*dIII and cloned into m13mp8 and m13mp9 to generate a coding-strand probe, mLvU1.1C, and a non-coding-strand probe, mLvU1.1NC.

Materials. Sea urchins (*L. variegatus*) were collected from the Gulf of Mexico and maintained at the Florida State University Marine Laboratory. *S. purpuratus* sea urchins were purchased from Pacific Biomarine (Venice, Calif.). α-Amanitin, CIP, γ-*S*-GTP, and γ-*S*-ATP were purchased from Boehringer Mannheim Biochemicals (Indianapolis,

Ind.). β-*S*-ATP was a generous gift of Michael Stallcup, University of South Carolina. Nitrocellulose (0.45 μm) was purchased from Schleicher & Schuell, Inc. (Keene, N.H.). [α-$^{32}PO_4$]CTP and [α-$^{32}PO_4$]GTP (>400 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, Mass.). S1 nuclease and restriction enzymes were purchased from Bethesda Research Laboratories.

RESULTS

Synthesis of U1 RNA in vitro. Nuclei from sea urchin embryos transcribe a number of different small RNAs in vitro (26). The small RNA products are conveniently prepared by removal of the nuclei from the transcription reaction by centrifugation through a glycerol pad. Over 90% of the small RNAs synthesized in vitro are found in the supernatant, and they can be analyzed directly by gel electrophoresis. Figure 1 shows the RNA synthesized and released from *S. purpuratus* and *L. variegatus* nuclei. Previously, we determined that the synthesis of one of the small RNAs which we detected in this manner was inhibited by a low concentration of α-amanitin (1 μg/ml) (26). The mobility of this RNA polymerase II product was similar to that of sea urchin U1 RNA (26).

To identify the RNA synthesized in vitro as authentic U1 RNA, the following experiments were done. The total RNA synthesized in vitro in the presence of low and high concentrations of α-amanitin was hybridized to cloned *L. variegatus* U1 DNA (pLvU1.1) immobilized on nitrocellulose (Fig. 1B). As controls, the same RNA was also hybridized to rDNA and histone DNA. The U1 RNA detected by dot hybridization showed the same sensitivity to α-amanitin as the RNA which comigrated with U1 RNA and histone mRNA. As expected, the synthesis of rRNA was not affected by α-amanitin. The RNA bound to the filter was eluted and analyzed by gel electrophoresis. The eluted RNA migrated as a single species colinear with U1 RNA (not shown, but see Fig. 4).

Initiation of sea urchin U1 RNA in vitro. We have calculated that sea urchin blastula embryos, to satisfy the requirement for synthesis of one of the most abundant RNAs in the cell, synthesize about one molecule of U1 RNA per gene per min (30). Given this high rate of expression and the fact that the RNA is small and should take less than a minute to transcribe, it seemed possible that at least a portion of the synthesis of this RNA in vitro resulted from de novo initiation, as has been found for the small RNA polymerase III transcripts (24, 36). To test the possibility of initiation, we used β-*S*- and γ-*S*-ribonucleotide triphosphates as substrates. These nucleotides are incorporated only into the 5′ nucleotide of the RNA (37). The initiated RNA containing thio groups can then be selected by chromatography on mercuriagarose. A possible complication of this approach for the U1 RNAs is that these RNAs have a 2,2,7-trimethyl-GpppA cap at their 5′ end. However, β-*S*-ATP could be incorporated into the cap structure, because the β-PO_4 of the initiating ATP is retained in the cap (14, 35). It is also possible that the thionucleotides are not good substrates for the phosphohydrolase or guanylyltransferase involved in the capping reaction. Thus, capping could be inhibited by thionucleotides as it is in reovirus (33). Because capping is not required for continued transcription in cell-free systems (6), it is unlikely that inhibition of capping would block transcription. A schematic of events which might occur, depending on the effect of thionucleotides on the different enzymatic steps, is shown in Fig. 2.

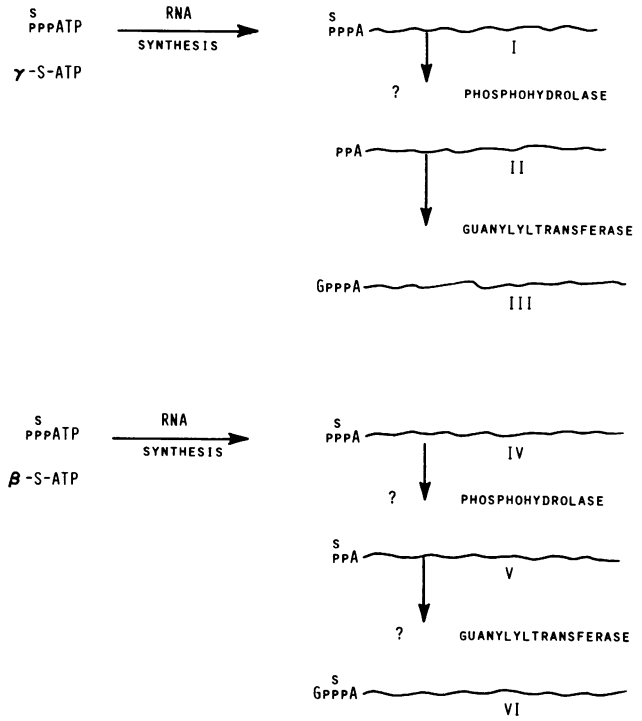

FIG. 2. Possible 5′ termini of RNAs initiated with thionucleotides. The possible 5′ structures formed with β-*S*-ATP or γ-*S*-ATP for initiation of RNA synthesis with RNA polymerase II are shown. The question marks indicate that it is not known whether the RNA containing thionucleotides is a substrate for the cellular phosphohydrolase or guanylyltransferase. If the phosphohydrolase is inhibited by β-*S*- or γ-*S*-ATP, then an uncapped transcript containing a thionucleotide would accumulate. If the guanylyltransferase is inhibited by a β-thionucleotide, then an uncapped transcript containing a thionucleotide would also accumulate. RNAs I, IV, V, and VI would be bound by mercuriagarose. After treatment of RNA with CIP, only RNA VI would be bound by mercuriagarose.

An additional potential problem with the use of thiophosphate nucleotides is the possibility that other reactions involving thionucleotides could alter the results. These might include randomization of the thionucleotide to other nucleotides (4) or the transfer of the thiophosphate to a previously initiated RNA. Transfer of thiophosphate to RNA might occur with γ-*S*-ATP but does not occur with β-*S*-ATP (39). Randomization of the label among different nucleotides at the γ-position would result in apparent lack of specificity in the initiating nucleotide.

Sea urchin nuclei were incubated with γ-*S*-ATP, γ-*S*-GTP, or β-*S*-ATP, together with [α-$^{32}PO_4$]CTP or [α-$^{32}PO_4$]GTP as the radiolabeled tracer. The total amount of RNA synthesized was lower in the presence of β-*S*-ATP as observed by Stallcup and Washington (38). The RNA in the released fraction was chromatographed on mercuriagarose. Similar percentages of the released RNA bound to the column (15 to 40%) with β-*S*-ATP and γ-*S*-ATP, although the amount of bound RNA varied with different nuclei preparations. The RNA in each fraction was analyzed by gel electrophoresis. Many different RNA species were bound to the mercuriagarose with β-*S*-ATP as the initiating nucleotide (Fig. 3A). Similar results were seen with γ-*S*-ATP (not shown). Included in these RNAs was an RNA which comigrated with sea urchin U1 RNA. No RNA with the mobility of sea urchin U1 RNA was synthesized if 1 μg of α-amanitin per ml was

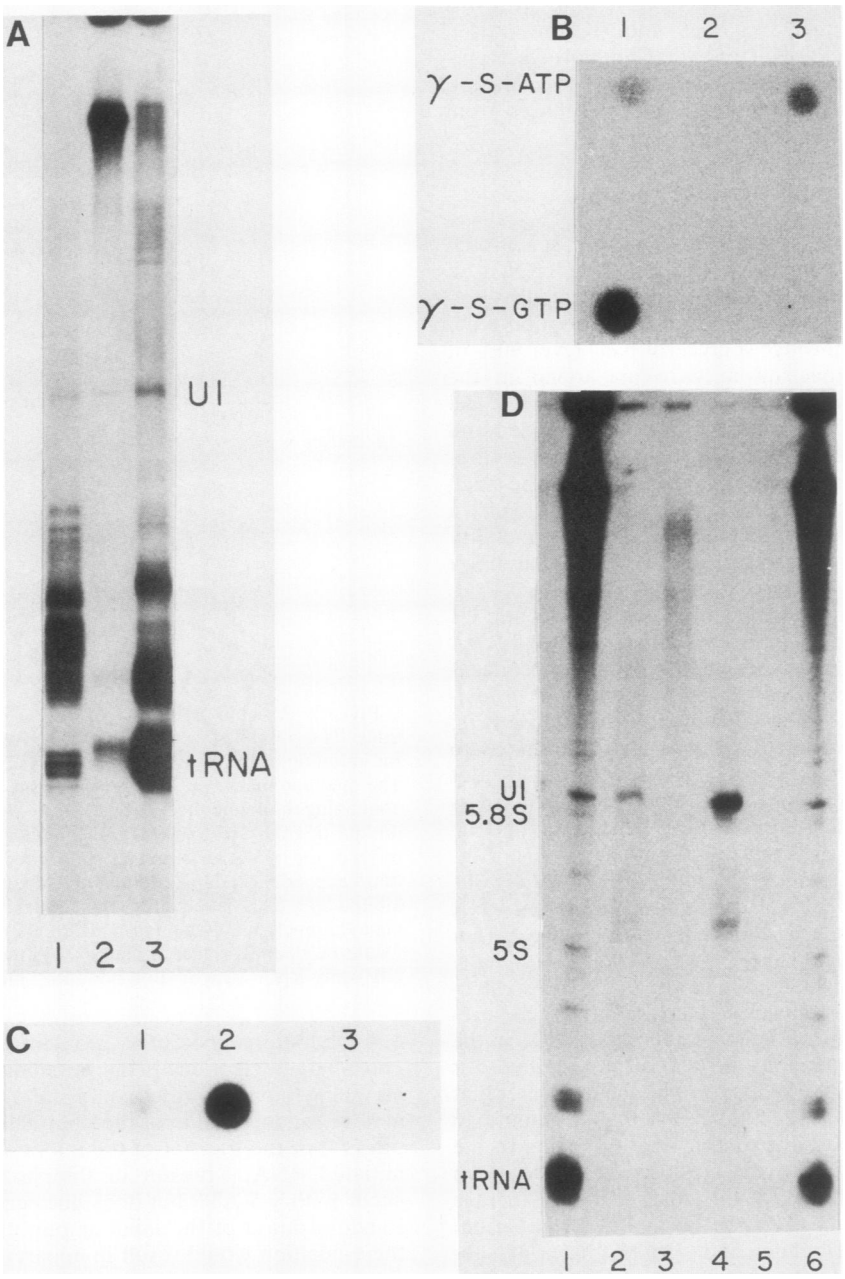

FIG. 3. Initiation of U1 RNA synthesis in isolated nuclei. (A) Nuclei prepared from *S. purpuratus* blastula embryos were incubated with β-*S*-ATP (lanes 1 and 3) and [α-$^{32}PO_4$]CTP. The released RNA was fractionated by chromatography on mercuriagarose. Equal amounts of the bound and unbound fractions were analyzed by gel electrophoresis. The RNAs which bound to mercuriagarose are shown in lane 1, and the RNAs which did not bind to the column are shown in lane 3. Control experiments demonstrated that there was no detectable binding of RNA if thionucleotides were not used as substrates. Lane 2 shows $^{32}PO_4$-labeled RNA prepared from *S. purpuratus* embryos. The extensive labeling of tRNA in lane 3 is the result of labeling of the CCA end of endogenous tRNA with [α-$^{32}PO_4$]CTP. (B) Nuclei from *S. purpuratus* embryos were incubated with γ-*S*-ATP or γ-*S*-GTP and [α-$^{32}PO_4$]CTP. The RNAs containing thionucleotides were selected by chromatography on mercuriagarose. The bound RNAs were hybridized to DNA immobilized on nitrocellulose. The DNAs used were 5S DNA, pLu305 (lane 1); mLvU1.1NC, the noncoding strand of the U1 gene cloned into m13mp8 (lane 2); mLvU1.1C, the coding strand of the U1 gene (nucleotides 30 to 140) cloned into m13mp9 (lane 3). (C) The same nuclei used in panel B were incubated with β-*S*-ATP, and the released RNA containing thionucleotides was selected by chromatography on mercuriagarose. The RNA was hybridized to DNA dots as described above. Lanes: 1, M13 DNA; 2, mLvU1.1C DNA; 3, mLvU1.1NC DNA. (D) The RNAs initiated with β-*S*-ATP (selected by chromatography on mercuriagarose), which hybridized to the coding and noncoding strands of the U1 gene, were eluted from the filter and analyzed by polyacrylamide gel electrophoresis. Lanes: 1 and 6, marker RNA; 2, RNA selected from the nuclear fraction by mLvU1.1C; 3, RNA selected from the nuclear fraction by mLvU1.1NC; 4, RNA selected from the released fraction by mLvU1.1C; 5, RNA selected from the released fraction by mLvU1.1NC. The band at about 120 nucleotides in lane 4 is the size of the insert of mLvU1.1C and possibly results from traces of nuclease activity during hybridization.

included in the reaction, although all the other RNAs observed in Fig. 3A were synthesized (data not shown). In three independent nuclear preparations, more of the U1 RNA was selected when β-*S*-ATP was used than when γ-*S*-ATP was used, suggesting that the phosphohydrolase may cleave the RNA initiated with γ-*S*-ATP at a low rate.

To confirm the identification of this band as U1 RNA, we did two types of experiments. First, the RNA was hybridized to DNA immobilized on nitrocellulose. Subclones coding for nucleotides 30 to 140 of the U1 RNA were constructed in phage M13. Separate clones coding for each strand of the DNA were used. Only the coding strand hybridized to RNA initiated with γ-*S*-ATP. As expected, the U1 M13 clone did not hybridize to RNA that was initiated with γ-*S*-GTP (Fig. 3B). In contrast, 5S DNA hybridized more strongly with the RNA initiated with γ-*S*-GTP, although hybridization to the cloned 5S DNA also occurred with RNA initiated with γ-*S*-ATP. This may be because a portion of the sea urchin 5S RNA is initiated with ATP (D. Stafford, personal communication). In a large-scale experiment, U1 RNA initiated with β-*S*-ATP was prepared by hybridization selection to the M13 clones (Fig. 3C). The RNA was eluted from the filter and analyzed by polyacrylamide gel electrophoresis (Fig. 3D). RNA with identical mobility with U1 was selected only by the coding strand.

The second experiment was an S1 nuclease mapping experiment (Fig. 4). The RNA initiated with β-*S*-ATP was hybridized with the cloned U1 DNA, pLvU1.1, or the same DNA cut with *Bgl*II at nucleotide 140. The hybrids were treated with S1 nuclease, and the resistant RNA was analyzed by gel electrophoresis. The entire gene protected an RNA which was the length expected for U1 RNA. The *Bgl*II-cut DNA protected an RNA of 140 nucleotides (A in Fig. 4, lane 3). These results map the 5′ end of the RNA synthesized in vitro to the same site as the RNA synthesized in vivo. We conclude that sea urchin blastula nuclei initiate and terminate the U1 RNA accurately.

To show that the RNAs made in vitro which bound to mercuriagarose actually had a thiophosphate at the 5′ termini, we treated the RNAs with CIP. This enzyme efficiently converts β-*S*-ATP and γ-*S*-ATP to adenosine (unpublished observations). After treatment of the thio-labeled RNA (purified by selection on mercuriagarose) with CIP, none of the RNA bound to mercuriagarose, indicating that the thiophosphate had been removed (Fig. 5). None of the RNA was degraded by the CIP treatment. If the RNA was rechromatographed on mercuriagarose without CIP treatment, it again bound to the mercuriagarose (data not shown). The same result was obtained when β-*S*-ATP or γ-*S*-ATP was used as a precursor. Because the 5′ end of the U1 RNA was completely sensitive to CIP, we conclude that the RNA initiated with thionucleotides was not capped (Fig. 2). Whether this result indicates that the nuclei are deficient in capping or that the thionucleotides inhibit capping is not known.

To assess whether the U1 RNA synthesized in vitro had identical mobility with the U1 RNA synthesized in vivo, we analyzed the RNAs on longer gels (Fig. 6). The U1 RNA migrated slightly faster than mature U1 RNA under these conditions (1 to 2 nucleotides). The U1 RNA which did not bind to the mercuriagarose had the same mobility as the RNA which contained the thiophosphate at the 5′ end. Removal of the 5′ phosphates did not affect the mobility of the U1 RNA of either fraction, although it clearly affected the mobility of some of the smaller transcripts, seen most readily on the lighter exposure of the gel (Fig. 6A, lanes 3 versus 6 and 4 versus 7). It is not clear what effect removal of the phosphates should have, because this reduces the size (increasing mobility) and decreases the charge (reducing mobility), as well as possibly influencing secondary structure. However, it is clear that the U1 RNA made in vitro is not identical to the in vivo RNA for unknown reasons, which could include base modifications (capping, methylation, pseudouridine formation) which might influence the secondary structure and hence the mobility. These long gels also clearly show that the small polymerase III transcripts (which are heterogeneous) initiated with β-*S*-ATP and bound to mercuriagarose are different from those which do not bind to the mercuriagarose (and which presumably are initiated with GTP). The identity of these bands is not known, but they probably include tRNA precursors as well as other small RNAs.

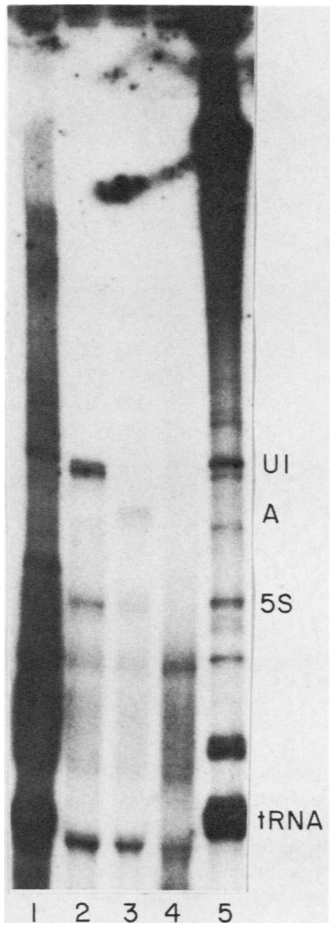

FIG. 4. S1 nuclease analysis of U1 RNA initiated with β-*S*-ATP. *S. purpuratus* nuclei were incubated with β-*S*-ATP and [α-$^{32}PO_4$]CTP. The released RNA was chromatographed on mercuriagarose. The bound RNA was hybridized with pLvU1.1 in solution. The hybrids were treated with nuclease S1, and the S1-resistant RNA was analyzed by gel electrophoresis. Lanes: 1, RNA bound to mercuriagarose; 2, S1-resistant RNA hybridized to pLvU1.1 cut with *Hin*dIII (the U1 gene is intact); 3, S1-resistant RNA hybridized to pLvU1.1 cut with *Bgl*II (the U1 gene is cut at nucleotide 140); 4, RNA bound to mercuriagarose treated with S1 nuclease; 5, marker RNA. The slightly faster mobility of U1 RNA in lane 2 is also seen when authentic U1 RNA is analyzed in the same way and is probably due to nibbling by S1 nuclease (5). A, See text for definition.

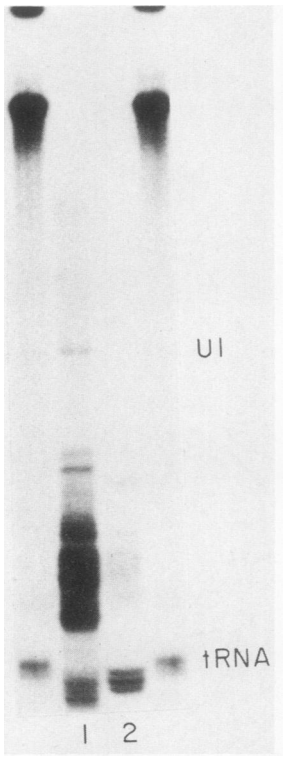

FIG. 5. Treatment of RNA with CIP. Nuclei from *S. purpuratus* embryos were incubated with β-*S*-ATP, and the released RNA was chromatographed on mercuriagarose. The bound RNA was treated with CIP and then rechromatographed on mercuriagarose. The bound (lane 2) and unbound (lane 1) fractions were analyzed by polyacrylamide gel electrophoresis. The outside lanes are marker RNAs.

DISCUSSION

Recent results in several systems indicate that all RNAs may be formed by RNA-processing reactions. The 5′ end of RNA polymerase II transcripts is capped, and the cap is formed by transfer of GMP to the initial nucleotide of the transcript after removal of the γ-phosphate (14). The one known exception to this mechanism is influenza virus which appropriates its caps from preformed cellular RNA (34). The snRNAs are the only known capped RNAs other than mRNAs, and the capping nucleotide is 2,2,7-trimethylguanosine rather than the 7-CH_3-guanosine found on mRNA.

The pathway of biosynthesis of snRNAs is not known. Several reports suggest that there may be precursors to the snRNAs at the 5′ end (10, 12). In a DNA-dependent system from HeLa cells, the sole transcript formed is initiated 183 bases 5′ to the first base of the mature RNA (29). This transcript has not been detected in vivo. The same U1 gene is initiated at base 1 when it is injected into *Xenopus* oocytes (35). In addition, there is a minor transcription start site at −110 in the *Xenopus* oocyte which yields a stable RNA which can be precipitated with anti-RNP antibody (35). It is likely these anomalous start sites at −110 and −183 are artifacts of the systems used.

U1 RNA synthesis in sea urchin nuclei. Transcription of sea urchin U1 RNA in isolated nuclei starts at base 1 of the RNA. The size of the U1 RNA synthesized is consistent with the 3′ end of the RNA also being formed accurately. Whether this results from transcription termination or

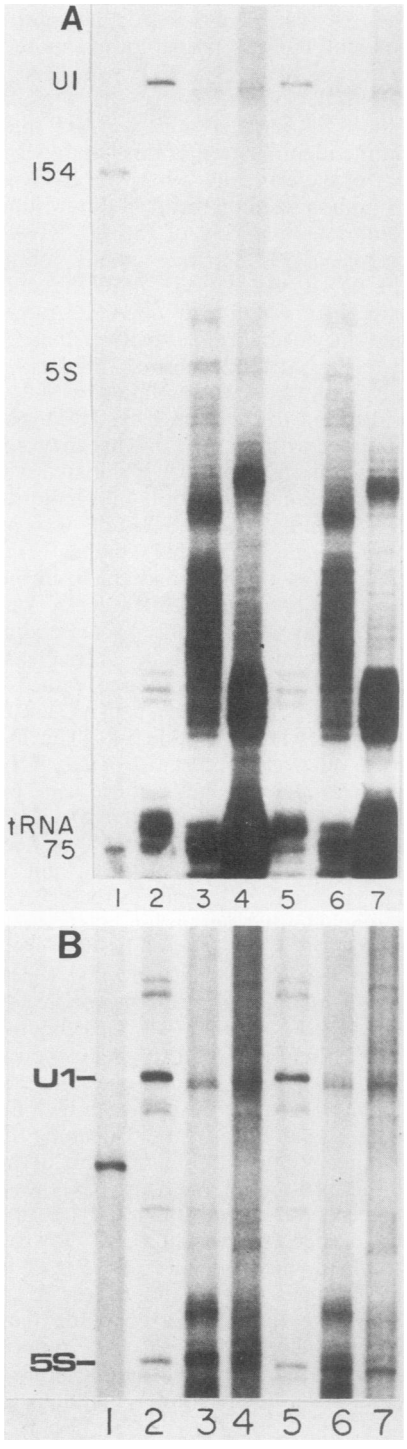

FIG. 6. High-resolution analysis of U1 RNA. (A) RNA synthesized in nuclei isolated from *S. purpuratus* embryos with β-*S*-ATP or synthesized in vivo was analyzed on a sequencing (35-cm) gel. Lane 1 is a DNA marker, a *Hin*fI digest of pBR322. The RNAs were analyzed before (lanes 2 to 4) and after (lanes 5 to 7) treatment with CIP. Lanes: 2 and 5, RNA labeled in vivo; 3 and 6, RNA synthesized in vitro which bound to mercuriagarose; 4 and 7, RNA synthesized in vitro which did not bind to mercuriagarose. RNA from equal amounts of nuclei was applied to each lane. (B) A longer exposure of the U1 RNA region of the gel shown in panel A.

processing is not known. RNA polymerase II generally does not terminate at the 3' end of RNAs which are going to be polyadenylated, but rather the transcript is cleaved before polyadenylation (16, 25). Nonpolyadenylated RNAs such as histone mRNAs also are formed by cleavage of the transcript at the 3' end (1, 32). The DNA sequences 3' to the end of the U1 RNA are very similar to those at the 3' end of the sea urchin histone genes (5, 15), suggesting that the 3' end is formed by a processing reaction.

Thionucleotides provide a sensitive tool for detecting initiation (36, 37). The snRNAs are particularly useful because of the relative abundance of transcripts and the ability to measure accurately the size of the RNA made in vitro. The transcription of U1 RNA is initiated relatively efficiently in the isolated nuclei. As much as 40% of the U1 RNA made in vitro is found in the fraction initiated in vitro.

Capping of U1 RNA in vitro. The ability of CIP to remove thiophosphates from nucleotides and RNAs allowed us to determine whether the U1 RNA synthesized in vitro was capped. We concluded that in sea urchin nuclei the phosphohydrolase does not cleave γ-thio-RNA efficiently and that neither the phosphohydrolase nor the guanylyltransferase will use the β-thio-RNA as a substrate. This is similar to the results obtained with reovirus (33) in which the reovirus phosphohydrolase was completely inhibited by γ-*S*-ATP. We consistently observed less U1 RNA initiated with γ-*S*-ATP than with β-*S*-ATP, consistent with the possibility that the sea urchin phosphohydrolase is able to remove the γ-thiophosphate, although at a low rate. The uncapped U1 RNA containing a thionucleotide accumulates stably in the reaction. We assume that the U1 RNA synthesized in the absence of thionucleotides is capped.

The U1 RNA synthesized in vitro migrates slightly faster during electrophoresis than does authentic U1 RNA. This could be due to one or more of several factors: failure to cap the RNA, failure to perform base modifications, or failure to form the 3' end accurately. Mobility is certainly influenced by the secondary structure of the RNA, even under the relatively denaturing conditions used. Any of the modifications mentioned above might affect the secondary structure.

Synthesis of other U-class RNAs. Of interest is the fact that these nuclei do not synthesize a detectable amount of RNA that comigrates with U2 RNA, although in vivo this RNA is synthesized at a rate about half that of U1 RNA (30). Mammalian nuclei also do not synthesize RNAs which comigrate with the mammalian U1 and U2 RNAs. Thus, the factors required for synthesis of other snRNAs may differ from those required for U1 synthesis and may be lost upon preparation of other nuclei. In this case, the snRNAs could accumulate as longer transcripts which would not be detected by gel electrophoresis. The rate of synthesis of U1 RNA varies during sea urchin development (30). This system should permit the study of factors which may influence initiation of U1 RNA synthesis as well as other events in U1 RNA metabolism, including base modification and formation of ribonucleoprotein particles.

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