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The cDNA sequences of the sea urchin U7 small nuclear RNA suggest specific contacts between histone mRNA precursor and U7 RNA during RNA processing

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3′ Processing of sea urchin H3 histone pre-mRNA depends on a small nuclear RNP which contains an RNA of nominally 60 nucleotide length, referred to below as U7 RNA. The U7 RNA can be enriched by precipitation of sea urchin U-snRNPs with human systematic lupus erythematosus antiserum of the Sm serotype. We have prepared cDNA clones of U7 RNA and determined by hybridization techniques that this RNA is present in sea urchin eggs at 30-fold lower molar concentration than U1 RNA. The RNA sequences derived from an analysis of eight U7 cDNA clones show neither homologies nor complementarities to any other know U-RNAs. The 3′ portion of the presumptive RNA sequence can be folded into a stem-loop structure. The 5′-terminal sequences would be largely unstructured as free RNA. Their most striking feature is their base complementarity to the 3′ conserved sequences of histone pre-mRNAs. Six out of nine bases of the conserved CAAGAAAGA sequence of the histone mRNA precursor and 13 out of 16 nucleotides from the conserved palindrome can be base paired with presumptive U7 RNA sequence, suggesting a unique hybrid structure for a processing intermediate formed from histone precursor and U7 RNA.

Key words: lupus antibodies/small nuclear RNP/U-RNA/sea urchin embryos/oocyte injection

Introduction

The eukaryotic cell contains a series of small RNPs, some of which are known to play a key role in cell metabolism. Thus, the U1 snRNP has been clearly implicated in the splicing of mRNA precursor molecules (Lerner *et al.*, 1980; Rogers and Wall, 1980; Yan *et al.*, 1981; Padgett *et al.*, 1983; Mount *et al.*, 1983; Krämer *et al.*, 1984). The eukaryotic RNase P, another snRNP (Akaboshi *et al.*, 1980), is required for tRNA maturation; the VA-RNA of cytoplasmic RNP particle, encoded by adenovirus (Mathews and Pettersson, 1978), modulates the translation efficiency of mRNAs (Thimmappaya *et al.*, 1982) while the Signal Recognition Particle is a component of a complex machinery mediating transmembrane transport of proteins (Walter and Blobel, 1980, 1982). The roles suggested for U4-snRNP in the polyadenylation of mRNA precursors (Berget, 1984) and U3-snRNP in ribosomal 5.8S and 28S maturation (Prestayko *et al.*, 1970) are interesting, but as yet unproven.

The 3′ editing of histone mRNA precursors (Birchmeier *et al.*, 1984; Krieg and Melton, 1984) is another well known cellular process which is coupled to a distinctive small nuclear RNP. Using an oocyte complementation assay, a 12S 'termination factor' (Stunnenberg and Birnstiel, 1982) containing a small RNA of nominally 60 nucleotide length, referred to below as U7 RNA, has been discovered which is an essential component for the maturation of a histone H3 pre-mRNA (Galli *et al.*, 1983) and probably of almost all other histone pre-mRNAs. The targets for the 'termination' snRNP are presumably sequences within the histone mRNA precursors. The most likely candidates are those sequences which have been shown to be required for the generation of faithful histone mRNA termini. These are: (i) the conserved hyphenated palindrome immediately upstream of the 3′ terminus (Birchmeier *et al.*, 1982, 1983, 1984), (ii) the conserved CAAGAAAGA six nucleotides downstream of the 3′ terminus (Georgiev and Birnstiel, 1985) and (iii) ~50 to 80 nucleotides succeeding spacer transcripts which have been shown to increase greatly the efficiency of 3′ processing (Birchmeier *et al.*, 1984; Georgiev and Birnstiel, 1985).

We have now prepared cDNA clones of U7 RNA, and have determined that this RNA is present in low abundance within the sea urchin cell. The U7 RNA can be enriched by precipitation of sea urchin U-snRNPs with human systemic lupus erythematosus antisera of the Sm serotype. The sequences of the U7 cDNA clones reveal striking complementarities between the 3′-terminal conserved sequences of the histone pre-mRNA and the 5′ sequences of the presumptive U7 RNA and suggest a unique hybrid structure for a processing intermediate formed from the histone RNA precursor and the U7 RNA.

Results

Sea urchin U7 RNA can be enriched by means of lupus antibodies of the Sm serotype

It became clear from our earlier work that the histone mRNA 'termination' factor and hence the U7 RNA must be present in the sea urchin in low concentration so that analysis of the U7 RNA would require extensive prior enrichment. We attempted to achieve this by both immunological and RNA separation techniques.

Antibodies of patients suffering from the systemic lupus erythematosus autoimmune disease of the Sm serotype (kindly provided by E. De Robertis, Basel), precipitate all U-snRNPs (except the U3 snRNP) (Lerner and Steitz, 1979) from a wide spectrum of species, including, for instance, *Xenopus* and *Drosophila* (Zeller *et al.*, 1983; Mount and Steitz, 1981). It seemed likely therefore that human Sm antisera might cross-react with sea urchin U-snRNPs and perhaps with our 'termination' factor (Stunnenberg and Birnstiel, 1982).

Antibodies were bound to protein A-Sepharose beads (De Robertis *et al.*, 1982) and reacted with homogenates of sea urchin ovaries. After extensive washing of the beads, RNA was recovered from the bound fraction by SDS treatment and deproteinization. The enrichment of the active RNA component was estimated by the oocyte complementation test described earlier (Galli *et al.*, 1983). This bioassay is based on the observation that processing of the sea urchin H3 pre-

mRNA synthesized from injected sea urchin histone DNA templates is exceedingly inefficient in the frog oocyte (Hentschel *et al.*, 1980), but that maturation of this RNA can be greatly accelerated by pre-injection of the U7 RNA into the oocyte (Galli *et al.*, 1983). In practice, RNA fractions are pre-injected into the cytoplasm of frog oocytes. It is presumed that the naked RNA is assembled into snRNP from pre-existing cytoplasmic proteins and that the snRNP then migrates into the nucleus (see De Robertis *et al.*, 1982). A day after injection of U7 RNA circularized cloned h22 DNA containing the five histone genes of *Psammechinus* is introduced into the nucleus. The degree of complementation of 3′ processing by U7 RNA is then quantified by Sl mapping (Berk and Sharp, 1977; Weaver and Weissmann, 1979) of the 3′ termini of the mature H3 mRNA. The 3′ terminus of H2A mRNA, whose maturation does not require added U7 RNA, provides an appropriate internal control.

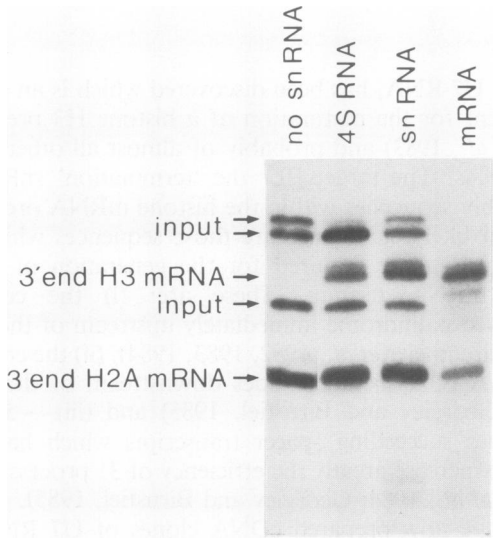

Fig. 1. Enrichment for U7 RNA by immunoprecipitation of RNPs with anti-Sm serum. 3′ Sl mapping of H3 and H2A transcripts from oocytes injected with H22 DNA circles and pre-injected with either 30 ng RNA of 4S sucrose gradient fraction/oocyte or 1.8 ng snRNA/oocyte. At this ratio of injected RNA (30:1.8) a similar degree of complementation of 3′ processing was obtained, hence the immunoprecipitated RNA was enriched for the active U7 RNA by a factor of 17.

'4S' sucrose gradient fractionated RNA or RNA prepared from immunoprecipitated ovarian snRNPs was injected at various concentrations into frog oocytes and the degree of complementation of H3 pre-mRNA 3′ processing was compared. In different experiments, RNA from immunoprecipitated snRNPs was 15- to 20-fold more active in generating H3 mRNA 3′ ends than the '4S' sucrose gradient fraction (Figure 1). If it is accepted that total snRNA represents ~5% of the '4S' RNA then a 20-fold purification of U7 RNA over '4S' RNA indicates that immunoprecipitation of U7 RNA as RNP is nearly quantitative.

An aliquot of the immunoprecipitated RNA was analyzed by labelling with [^{32}P]pCp and RNA ligase (England and Uhlenbeck, 1978) and separation on a sequencing gel. The autoradiogram revealed in the upper half a predominance of the U-RNAs similar to those known from mammalian species (Figure 2a). RNA species smaller than 100 nucleotides were thought to represent, in the main, degradation products of these U-RNAs. Faint RNA bands with an estimated length of

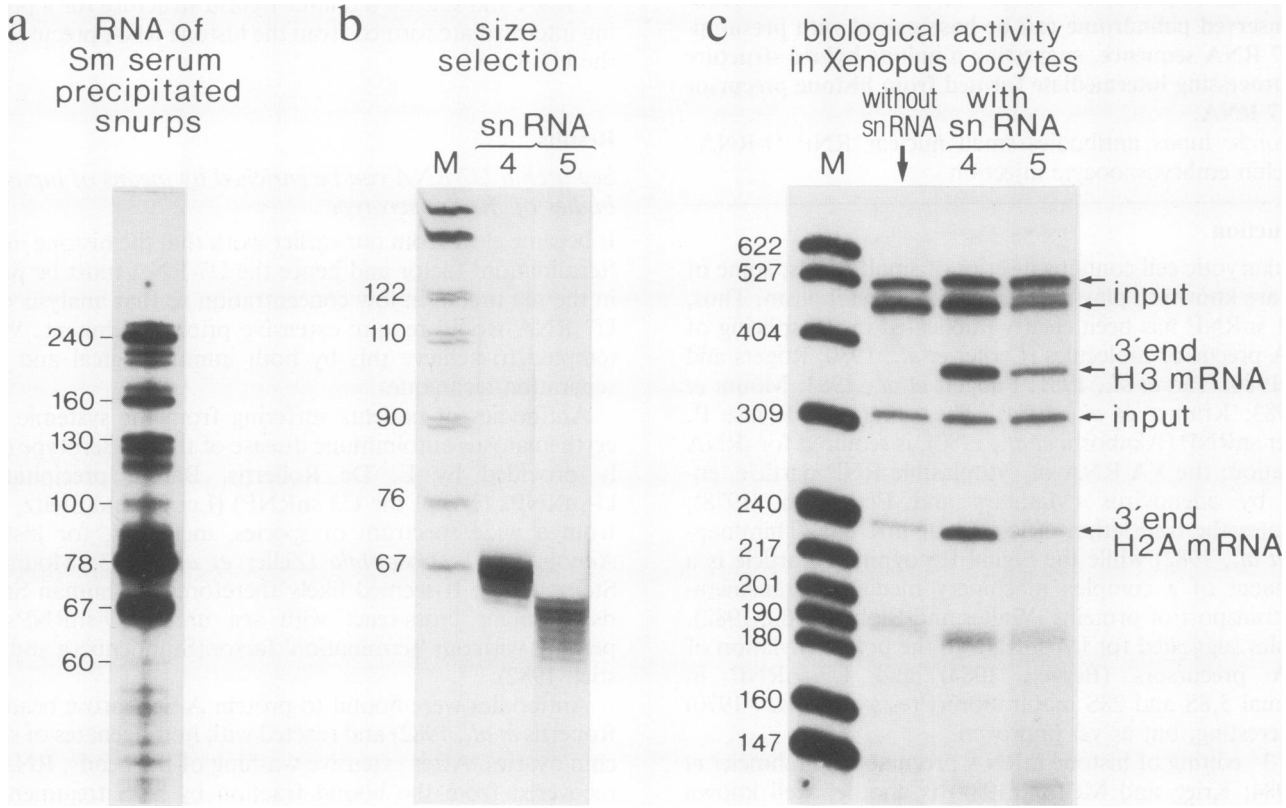

Fig. 2. Immunoprecipitation and fractionation of snRNA. **(a)** Autoradiogram of a 8 M urea 15% polyacrylamide gel of total [^{32}P]pCp-labelled RNA extracted from immunoprecipitated snRNPs of sea urchin ovaries. **(b)** End-labelled RNA fractions recovered from a sliced preparative sequencing gel of total snRNA. **(c)** 3′ Sl mapping of H3 and H2A transcripts from *X.laevis* oocytes that were injected into the cytoplasm either with injection buffer or size selected snRNA (fractions 4 and 5) prior to nuclear injection with h22 DNA circles.

50–60 nucleotides were also seen on the gel. In order to purify further the active component the snRNA was fractionated on a 15% polyacrylamide sequencing gel. After ethidium bromide staining RNA molecules of the 50–70 nucleotide size class were recovered from RNA gel slices. The reclaimed RNA was radioactively labelled and rerun on an analytical gel (Figure 2b). Both fraction 4 and 5 were seen to be polydisperse and to lead to the appearance of H3 mRNA 3′ termini when assayed in the oocyte (Figure 2c) whereas flanking fractions proved to be inactive (results not shown).

Cloning of cDNA prepared by reverse transcription of U7 RNA

At this stage of the analysis, it was self-evident that U7 RNA could not be purified to homogeneity and in sufficient amounts for direct RNA sequencing by the above method. Therefore, it was decided to prepare cDNA clones from the still polydisperse RNA fractions and to identify the relevant clones by the use of our bioassay system. This procedure ensures that the biologically active component(s) will be selected from the prevalent RNA degradation products.

In short, fraction 4 of Figure 2c was first polyadenylated by *Escherichia coli* adenylate transferase (Sippel, 1983) and then reverse-transcribed into cDNA by standard procedures (Land *et al.*, 1981). The cDNA was freed from the RNA by alkali treatment and tailed with dTTP. After priming with oligo(dA) the second strand of the DNA was synthesized with reverse transcriptase. The now double-stranded DNA was tailed once more with dTTP to create poly(T) overhangs and the product annealed to poly(A)-tailed pSP64 plasmid DNA (see Figure 3).

cDNA clones were screened for U7 sequences by hybridization of 4S RNA to filter-bound cDNA clones (in groups of 12), followed by injection of hybrid-selected RNA into the frog oocyte. As can be seen from Figure 4, RNA selected by DNA pool 22 was capable of eliciting the generation of genuine 3′ H3 mRNA termini in high yield, whereas RNA selected by DNA pools 13 and 25 was not active. The DNA clones of pool 22 were then tested individually. In this way a cDNA clone containing the U7(7) sequence of Figure 5 was obtained. A DNA sequence complementary to the last 15 nucleotides of the U7(7) RNA was synthesized, ^{32}P end-labelled and used as a hybridization probe to identify another nine U7 cDNA clones by the Grunstein-Hogness colony hybridization procedure (Grunstein and Hogness, 1975) out of a total of 600 colonies. The 10 cDNA inserts were then sequenced by the Maxam and Gilbert procedure (Maxam and Gilbert, 1977). Once analyzed, the U7 RNA-like DNA strand could be easily identified due to the asymmetrical tails which were generated during cDNA cloning (Figure 3).

Abundance of U7 RNA in the sea urchin

The immunoprecipitation, RNA fractionation and cDNA cloning experiments all suggested that U7 RNA was a rare RNA species. We therefore decided to measure its concentration in total sea urchin RNA by hybridization with *in vitro* synthesized anti-sense U7 RNA. In previous cloning experiments we identified a pSP64-cDNA clone which contained the 3′-terminal 59 nucleotides of the U1 RNA of the sea urchin *Strongylocentrotus doebrachiensis*. Complementary [^{32}P]RNA of known specific activity was prepared by *in vitro* transcription of the U1 and U7 cDNA clones (Green *et al.*, 1983) and the RNA hybridized in excess with sea urchin egg RNA. The relative concentration of U1 and U7 RNA was determined by hybridization (Zinn *et al.*, 1983). The RNA

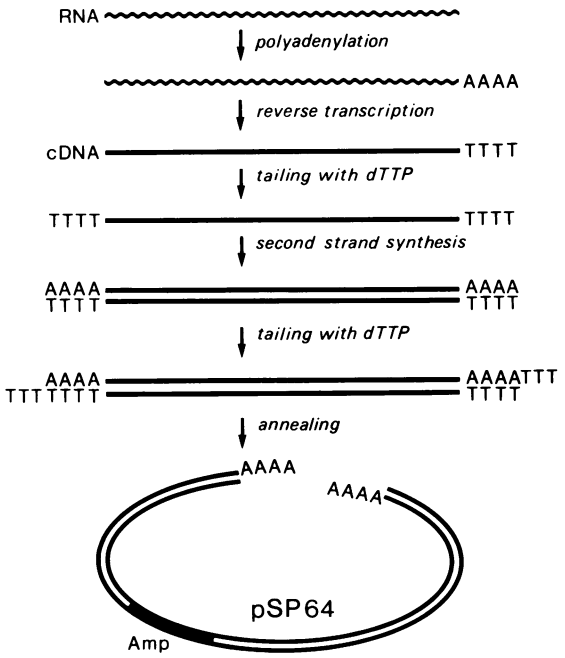

Fig. 3. Scheme for cDNA cloning of U7 RNA

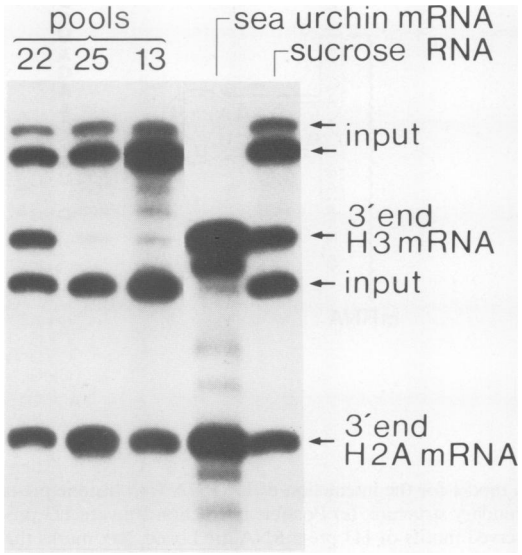

Fig. 4. Screening for U7 cDNA clones by testing the biological activity of hybrid-selected RNA. 3′ S1 mapping of H3 and H2A transcripts from oocytes injected with h22 DNA circles and pre-injected with either 4S RNA or RNA hybrid-selected from different cDNA pools, each pool containing 12 individual cDNA clones.

species were found to be present in sea urchin egg RNA in a molar ratio of 30:1 (results not shown).

RNA sequence predicted from analysis of the cDNA clones

Of the 10 cDNAs analyzed by sequencing, five extend over 56 nucleotides (Figure 5). One clone contains an extra T at the 3′ terminus and therefore is 57 nucleotides long. Others gave partial sequences as shown for U7(7) and U7(8) cDNA. These provide truncated U7 versions which may be useful in later analyses of U7 RNA function. The sequences show a small degree of polymorphism, particlarly at position 30. The U content varies between 25 and 28%.

When inspecting the presumptive RNA sequences it

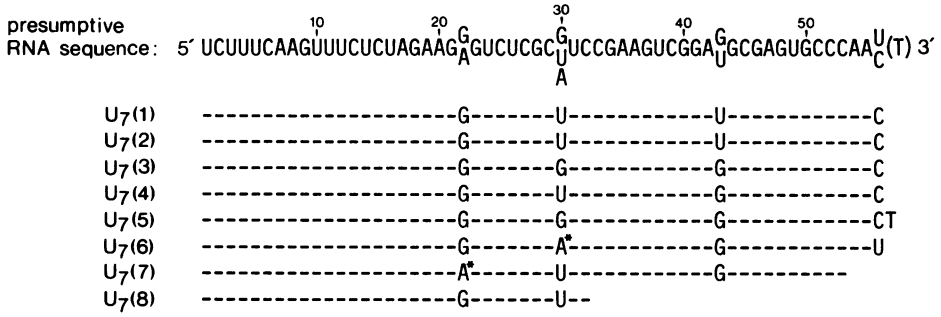

Fig. 5. Primary structure of U7 RNA deduced from cDNA sequences. The A residues marked with an asterix might have been generated by de-amination of C during alkali treatment of the cDNA during cloning.

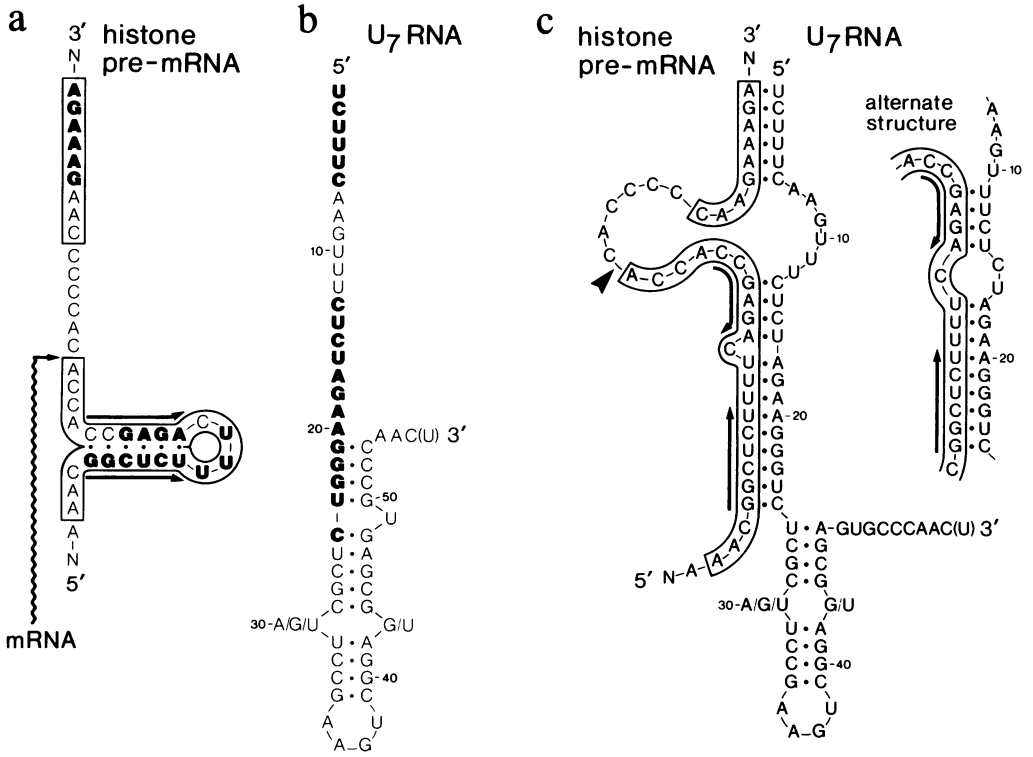

Fig. 6. A model for the interaction of U7 RNA with histone pre-mRNA. **(a)** Sequence of precursor RNA flanking the 3′ end of H3 mRNA. **(b)** Predicted U7 RNA secondary structure. **(c)** Possible interaction between H3 pre-mRNA and U7 RNA. Nucleotides interacting with each other are written in bold face type. The conserved motifs of H3 pre-mRNA are boxed. ➤ marks the putative 3′ end of the mature histone mRNA and the arrows indicate the nucleotides involved in the palindrome.

becomes immediately clear that the nucleotide sequences from position 21 to 53 can be folded into a palindrome containing at most one mismatched base pair and one looped out uridine residue (Figure 6b). The palindrome has a calculated increment of free energy of −24 kcal/mol (Tinoco *et al.*, 1973). There is also a potential palindrome made up from the RNA sequence positions 11−23, which would be thermodynamically unstable a free RNA.

The most striking feature of the derived RNA sequence is the sequence complementarity between the 5′ portion of the U7 RNA and the histone pre-mRNA sequences near the 3′ terminus. The 5′ most nucleotides of the U7 RNA can be matched up with the conserved CAAGAAAGA box (Busslinger *et al.*, 1979), whereas the U7 RNA sequence from positions 13−25 is complementary to the histone mRNA terminal palindrome (Busslinger *et al.*, 1979; see Discussion).

Discussion

Does the novel small nuclear RNP belong to the family of U-snRNPs?

The RNA sequence derived from sequencing a series of U7 cDNA clones demonstrates that the small RNA required for H3 pre-mRNA maturation is a unique RNA species. There are no significant sequence homologies to any other known U-RNAs, nor does the sea urchin U7 RNA contain sequences complementary to these RNA, as is the case between U4 and U6 RNA (Hashimoto and Steitz, 1984). This conclusion must be tempered by the knowledge that other sea urchin U-RNA sequences are not as yet available and that the above sequence comparison has therefore been made with vertebrate U-RNAs (Reddy and Busch, 1983) whose sequence divergence from sea urchin RNAs is unknown. In this context

it should be noted that the short U1 segment cloned in this laboratory from a sea urchin has a 22% base sequence divergence compared with the homologous U1 RNA segments of man (unpublished results). Nevertheless, U7 cDNA clones (containing both RNA-like and RNA-coding strand) when challenged with sea urchin '4S' RNA or [^{32}P]pCp-labelled RNA from immunoprecipitated sea urchin snRNPs hybrid-selected only U7 and no other U RNAs (unpublished results). This supports the notion that the U7 RNA is not a derivative of some other U-RNAs and that it contains no extensive sequence complementarities to other U-RNA species.

Sm antibodies recognize and precipitate snRNPs by interacting with the protein moiety (Lerner and Steitz, 1979; Billings and Hoch, 1983) rather than the RNA. The observation that U7 RNA can be purified from cell homogenates by anti-snRNP antibodies of the Sm serotype lends further credence to our previous postulate that U7 is contained in an RNP within the sea urchin cells (Galli *et al.*, 1983). The straightforward interpretation of the immunological results is that the U7 snRNP shares some protein(s) in common with other U-snRNPs, but the possibilities have not entirely been excluded that U7 snRNPs are co-precipitated with other RNPs because of a fortuitous association with them or because the polyclonal Sm antiserum contains an as yet uncharacterized determinant. An analysis of the protein(s) associated with the U7 RNA will help to clarify the situation.

U-RNAs of vertebrates, as suggested by their name, have a high content in uridilic acid, usually of the order of 30% (reviewed by Reddy and Busch, 1983). With an U content as high as 28% [as in U7(1) and U7(2) of Figure 5] sea urchin RNAs appear to conform to this general rule. Furthermore, like U1 RNAs (Lerner *et al.*, 1980; Rogers and Wall, 1980; Yang *et al.*, 1981; Padgett *et al.*, 1983; Krämer *et al.*, 1984), U7 RNA is involved in the editing of mRNA precursors (Galli *et al.*, 1983). U7 RNPs can be precipitated with anti-Sm antibodies (but see caveat above). Thus, there are a series of features common to U1 – U6 and U7 RNAs and their RNPs which suggest that the U7 snRNP should tentatively be classified as a genuine, if sparsely represented, member of the U-snRNP family.

The presumptive sequence of U7 RNA

U7 RNA was previously established as having a length of ~60 nucleotides (Galli *et al.*, 1983). The longest cDNA sequence U7(5) contains 57 nucleotides while in five other cases cDNAs extended to 56 nucleotides. In every case these inserts are flanked by runs of poly(A). Becasue of the tailing of the RNA and cDNA during cloning, it remains uncertain whether any of these adjoining As are genuine components of the U7 RNA or simply tailing products. The exact size of U7 RNA remains to be established.

The analysis of the cDNAs suggests a small degree of sequence polymorphism specially at position 30 as shown in Figure 5. A change from G to A could possibly be the result of de-amination of C during alkali treatment of the cDNA during the cloning procedure but G-U exchanges could not be explained in this way. A small portion ($\leq$11%) of the nucleotides of known U-RNAs are either methylated or converted to pseudo-uridine (reviewed by Reddy and Busch, 1983). The effects of such modifications on cDNA synthesis are not known but the presence of modified nucleotides in the RNA template could be a source of inaccuracy in the cDNA sequence and simulate a polymorphism where there is none. Analysis of the RNA will be required to resolve the problems listed above, although the difficulties of sequencing a 60 nucleotide RNA which is present in such low concentration will present a considerable logistic problem. Despite these limitations, several predictions can be made at least as to the gross features of the U7 RNA and its possible mode of action during histone mRNA 3′ processing.

A predicted U7 RNA structure

The U7 nucleotide sequence from position 21 to 53 is largely self complementary and would be expected to form a palindrome in RNA. It has been calculated that the increment in free energy of this palindrome would be at least −24 kcal/mol. Positions 30 and 43 show base mismatches in most, but not all, derived RNA sequences. These positions correspond to two out of the four sites where sequence polymorphism is suggested by cDNA sequencing. Only in the U7(4) and U7(7) RNA can nucleotides at positions 30 and 43 be base paired, by adposition of a U and a G. Quite possibly these clones reflect the true situation for U7 RNA. The U at position 49 is looped out from the double-stranded RNA stem in all available U7 RNA sequences.

The 5′ portion of U7 RNA would, on the whole, not be expected to form a folded structure, although the sequences from positions 11 to 23 could form a palindrome, the 3′ hairpin loop is partially destabilized. The most striking feature of the sequence from positions 1 to 25 is its complementarity to known conserved sequence blocks in the histone RNA precursors. This parallels the situation for the U1 RNA where the 5′-terminal sequences are also thought to interact with the acceptors sites of splice junctions by their base complementarity (Padgett *et al.*, 1983; Yang *et al.*, 1981). The 5′-terminal sequences of the U1 RNA are accessible enough in the RNP particle to be able to hybridize to a synthetic complementary DNA-oligonucleotide (Rinke *et al.*, 1984; Krämer *et al.*, 1984) to be digested with micrococcal nuclease (Reveillaud *et al.*, 1984) while other portions of the RNA are thought to interact with structural proteins (discussed by Brunel *et al.*, 1984). It is possible that the U7-snRNP is organized along similar lines with the 5′ portion of the U7 RNA being unstructured, largely protein-free and accessible for hybridization with pre-mRNA, while the 3′ portion of the molecule might provide an anchor for structural protein(s).

A model for the interaction of U7 RNA and histone pre-mRNA

In sea urchin histone genes the 3′ termini of the mRNA coding sequences are flanked by two conserved sequence blocks, the palindrome and the CAAGAAGA sequence which are themselves separated by exactly six nucleotides of an evolutionarily divergent sequence (Busslinger *et al.*, 1979). It has been clear for some time that the terminal stem-loop structure is essential for histone mRNA maturation (Birchmeier *et al.*, 1983, 1984) and that it acts at the level of RNA, not DNA (Birchmeier *et al.*, 1983, 1984; Georgiev and Birnstiel, 1985). In contrast to functionally important promoter sequence blocks, where the topology (and even orientation) can be altered in relatively wide limits without affecting promoter strength (Grosschedl and Birnstiel, 1980; McKnight and Kingsbury, 1982; Everett *et al.*, 1983) small deletions and sequence insertions between the two conserved sequence blocks immediately abolish all histone mRNA 3′ processing (Georgiev and Birnstiel, 1985). These two sequence blocks, although separated by evolutionarily divergent sequences in the histone pre-mRNA, should be viewed therefore as a single

RNA element of defined topology directing 3′ RNA processing (Georgiev and Birnstiel, 1985).

One of these blocks, CAAGAAAGA, is a sequence element which is conserved in the histone genes of the sea urchins, but the other sequence block, the terminal mRNA palindrome, possesses the remarkable feature that its sequence conservation, in species as different as sea urchin and *Drosophila* to man, extends to even the unmatched bases in the hyphen of the palindrome (see Table I of Birchmeier *et al.*, 1983). The comparison of U7 RNA and the histone pre-mRNA sequences now provides possible explanations for these observations.

In discussing possible hybrid structures we shall restrict ourselves in this paper to the interaction of pre-H3 mRNA and the U7 RNA which was selected by our bioassay. As seen in Figure 6c, six out of nine bases of the conserved CAAGAAAGA sequence and 13 out of the 16 nucleotides out of the conserved palindrome can be base paired with U7 RNA. Note that in this hypothetical scheme the terminal palindrome has been linearized and that three out of four bases in the palindrome loop are annealed with U7 RNA. This leaves the conserved C of the hyphen in an unmatched configuration. U7(7) RNA fits the pre-mRNA sequence best because it has an A at position 22 (Figure 5), while in all other cases we have an inferred U-G pairing at this position.

Studies of complexes between oligonucleotides and complementary anticodon loops or between tRNAs with complementary anticodons have revealed that short sequences of as little as three nucleotides can form stable hybrids if these are presented in the loop of a palindrome (Grosjean *et al.*, 1976 and references therein). We know from mutagenesis experiments that the terminal histone mRNA sequences support RNA processing only when they are in a hairpin configuration (Birchmeier *et al.*, 1984). In such a spatial arrangement the highly conserved U-U-U-C sequence of the palindrome loop, containing three nucleotides complementary to U7 RNA, might provide, in an energetically favored way, the initial nucleation for the hybridization reaction which would then be followed by zippering of the adjacent complementary sequences. The entire hybrid complex, as shown in Figure 6c, would contribute −31 kcal/mol towards the stability of the structure as compared with an increment of free energy of the pre-mRNA folding of −10.6 kcal/mol (Busslinger *et al.*, 1979) and of U7 RNA folding of −24 kcal/mol.

The complex in Figure 6c contains some unmatched bases and single-stranded loops. Of particular interest is the nucleotide C of the palindrome loop which is conserved in the histone genes of most species (Birchmeier *et al.*, 1983) and yet it is unmatched. Such 'flaws' in RNA duplex structures can be of functional importance as exemplified by the interaction of a translational inhibitor with its target site on phage R17 RNA (Uhlenbeck *et al.*, 1983). Because of the distribution of complementary regions on the two nucleotide chains, hybrid formation between U7 and pre-mRNA forces the 3′-terminal histone mRNA sequences and the adjacent evolutionarily divergent pre-mRNA sequence into a relatively large single-stranded loop in which the mRNA 3′ terminus will eventually be generated.

From the data presented here, we have been able to predict a possible mechanism of interaction between H3 pre-mRNA and U7 RNA. However, in previous studies we have found that the spacer transcripts downstream of the CAAGAAAGA block are also required for establishing a maximal rate of 3′ processing. The mechanistic role of these spacer sequences is at present unclear. It is noteworthy that these stretches complementary to U7 RNA are essentially those held in common between sea urchin mRNA precursors, while the additional spacer sequences involved in RNA maturation have diverged rapidly in evolution; it therefore seems that they must exert their influence on maturation by some means other than base pairing to U7 RNA (Georgiev and Birnstiel, 1985).

The structural analyses of the cDNA clones make clear predictions as to molecular intermediates and possible mechanisms for the generation of 3′ termini of histone mRNA by RNA processing. These ideas will have to be tested by sequencing of the U7 RNA itself, by construction of complimentary mutants in both U7 and pre-mRNA and co-expression of these mutants in the living cell. In addition, the U7 snRNP will have to be characterized biochemically and its mode of action investigated by the use of available *in vitro* processing systems (Price and Parker, 1984; D. Schümperli, unpublished results).

Materials and methods

Lupus antisera was kindly provided by Dr. De Robertis (Basel), *E.coli* adenylate transferase by Professor Billeter (Zürich), calf thymus terminal deoxynucleotide transferase by Professor Weissmann (Zürich) and plasmid pSP64 by Dr. Melton (Boston). Avian myeloblastosis virus (AMV) reverse transcriptase was obtained from Life Science.

Immunoprecipitation and fractionation of snRNA

Ovaries of *P. miliaris* were homogenized and then sonicated in phosphate buffered saline (PBS) (130 mM NaCl, 34 mM sodium phosphate pH 7.4). Immunoprecipitation of snRNP with anti-Sm lupus erythematosus antibodies was performed as described by De Robertis *et al.* (1982). All procedures were carried out at 4°C. The extracted snRNA was fractionated on a 8 M urea, 15% polyacrylamide gel. RNA of different size classes ranging from 50 to 70 nucleotides were extracted, phenolized, precipitated and redissolved in 10 mM Tris, 1 mM EDTA. The biological activity of individual fractions was tested in *X.laevis* oocytes by microinjection (Galli *et al.*, 1983). All preparations were analyzed by end-labelling with 5′ [^{32}P]pCp and T4 RNA ligase (England and Uhlenbeck, 1978) followed by separation on 8 M urea, 15% polyacrylamide gels.

cDNA cloning of U7 RNA

In a first step, a biologically active snRNA fraction (300 ng) was polyadenylated with *E.coli* adenylate transferase according to Sippel (1983) prior to cDNA cloning which was performed as described in detail by Land *et al.* (1981) except for some modifications. The polyadenylated snRNA was transcribed into cDNA with AMV reverse transcriptase (40 units) using 1 μg of oligo(^{dT}12−18) as primer. After hydrolysis of the RNA in 0.2 M NaOH at 42°C for 1 h, the cDNA was freed of excess primer by separation on a 7 M urea, 8% polyacrylamide gel. The gel-eluted cDNA was elongated at the 3′ end with dTMP by terminal deoxynucleotide transferase from calf thymus prior to second strand synthesis with AMV reverse transcriptase and oligo(dT_{12-18}) as primer. At the end of the reaction the double-stranded cDNA was separated from unreacted primer on Sephadex G150 in 10 mM Tris pH 7.5, 1 mM EDTA. Following a further tailing step with dTTP and terminal deoxynucleotide transferase, the cDNA was annealed at room temperature in 0.5 M NaCl to pSP64 plasmid DNA previously poly(A)-tailed at the *Pst*I site and was then transfected into *E.coli* strain SK 1592 (Peacock *et al.*, 1981).

Identification of U7 cDNA clones

Individual cDNA clones were grown overnight at 37°C in 8 ml of L-broth containing 50 μg/ml of ampicillin. DNA was isolated from 12 pooled cultures by the clear lysate method. One half of each DNA preparation was linearized by *Eco*RI and the other half by *Hind*III, the two digestions were combined, extracted with phenol and precipitated with ethanol. This linearized DNA was resuspended in 1 ml of 20 mM EDTA, 10 mM Tris-HCl pH 7.5. After addition of an equal volume of 1 M NaOH these samples were incubated at room temperature for 20 min and then neutralized with 13 ml of a solution containing 1 M NaCl, 0.1 M soldium citrate 0.05 M Tris-HCl at pH 8.0 and 0.1 M HCl. The DNA was immediately bound to nitrocellulose filters. Filters were washed with 0.9 M NaCl, 0.09 M sodium citrate and baked for 3 h at 80°C in a vacuum oven. Filter hybridization and RNA elution was performed according to the method of Ricciardi *et al.* (1979). 2 mg of a sucrose gradient 4S fraction of total *P.miliaris* RNA from ovaries were hybridized to the filter-

bound DNA in a single polypropylene tube containing 3.5 ml hybridization solution consisting of 10% dextran sodium sulfate, 0.2% SDS, 0.9 M NaCl, 0.09 M sodium citrate at 50°C overnight. The filters were then washed three times for 30 min in 0.15 M NaCl, 0.01 M sodium citrate at room temperature. For elution of hybridized RNA the filters were transferred to glass vials (a pool of 12 clones in each tube) containing 400 μl of 10 mM Tris-HCl pH 7.0 and 1 μg *Xenopus* oocyte RNA. The tubes were placed in boiling water for 2 min, quick frozen in a dry ice/ethynol bath and thawed on ice. The filters were removed and the eluted RNA was extracted with phenol/chloroform, ethanol-precipitated and resuspended in 5 μl of oocyte injection buffer (88 mM NaCl, 10 mM Tris). This hybrid-selected RNA was tested for its biological activity by microinjection in *Xenopus* oocytes as described by Galli *et al.* (1983). cDNA inserts were sequenced from restriction sites in the polylinker of the plasmid pSP64 by the Maxam and Gilbert procedure (Maxam and Gilbert, 1977). Some U7 cDNA clones were identified by colony hybridization with a 15 nucleotide long oligonucleotide complementary to the 3′ end of U7 RNA (synthesized by Dr. K. Kalusa, ETH Zürich) in 6 x NET (1 x NET = 0.15 M NaCl, 0.015 M Tris/HCl pH 7.5, 0.001 M EDTA), 10% dextran sulfate at 49°C overnight. The filters were washed in 6 x NET at 37°C.

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References

Akaboshi,E., Guerrier., Takada,C. and Altman,S. (1980) *Biochem. Biophys. Res. Commun.*, **96**, 831-837.
Berget,S.M. (1984) *Nature*, **309**, 179-182.
Berk,A.J. and Sharp,P.A. (1977) *Cell*, **12**, 721-732.
Billings,P.B. and Hoch,S.O. (1983) *J. Immunol.*, **131**, 347-357.
Birchmeier,C., Grosschedl,R. and Birnstiel,M.L. (1982) *Cell*, **28**, 739-745.
Birchmeier,C., Folk,W. and Birnstiel,M.L. (1983) *Cell*, **35**, 433-440.
Birchmeier,C., Schümperli,D., Sconzo,G. and Birnstiel,M.L. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 1057-1061.
Brunel,C., Sri Widada,J. and Jeanteur,P. (1984) *Progr. Mol. Subcell. Biol.*, in press.
Busslinger,M., Portmann,R. and Birnstiel,M.L. (1979) *Nucleic Acids Res.*, **6**, 2997-3008.
De Robertis,E.M., Lienhard,S. and Parisot,R.F. (1982) *Nature*, **295**, 572-577.
England,T.E. and Uhlenbeck,O.C. (1978) *Nature*, **275**, 560-561.
Everett,R.D., Baty,D. and Chambon,P. (1983) *Nucleic Acids Res.*, **11**, 2447-2464.
Galli,G., Hofstetter,H., Stunnenberg,H.G. and Birnstiel,M.L. (1983) *Cell*, **34**, 823-828.
Georgiev,O. and Birnstiel,M.L. (1985) *EMBO J.*, **4**, in press.
Green,M.R., Maniatis,T. and Melton,D.A. (1983) *Cell*, **32**, 681-694.
Grosjean,H., Söll,D.G. and Crothers,D.M. (1976) *J. Mol. Biol.*, **103**, 499-519.
Grosschedl,R. and Birnstiel,M.L. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 7102-7106.
Grunstein,M. and Hogness,D.S. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 3961-3965.
Hashimoto,C. and Steitz,J.A. (1984) *Nucleic Acids Res.*, **12**, 3283-3293.
Hentschel,C.C., Probst,E. and Birnstiel,M.L. (1980) *Nature*, **288**, 100-102.
Krämer,A., Keller,W., Appel,B. and Lührmann,R. (1984) *Cell*, **38**, 299-307.
Krieg,P.A. and Melton,D.A. (1984) *Nature*, **308**, 203-206.
Land,H., Grez,M., Hauser,H., Lindenmaier,W. and Schütz,G. (1981) *Nucleic Acids Res.*, **9**, 2251-2266.
Lerner,M.R. and Steitz,J.A. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 5495-5499.
Lerner,M.R., Boyle,J.A., Mount,S.M., Wobis,S.L. and Steitz,J.A. (1980) *Nature*, **283**, 220-224.
Mathews,M.B. and Pettersson,U. (1978) *J. Mol. Biol.*, **119**, 293-328.
Maxam,A.M. and Gilbert,W. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 560-564.
McKnight,S.L. and Kingsbury,R. (1982) *Science (Wash.)*, **217**, 316-324.
Mount,S.M. and Steitz,J.A. (1981) *Nucleic Acids Res.*, **9**, 6351-6368.
Mount,S.M., Petterson,I., Hinterberger,M., Karmas,A. and Steitz,J.A. (1983) *Cell*, **33**, 509-578.
Padgett,R.A., Hardy,S.F. and Sharp,P.A. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 5230-5234.
Peacock,S.L., McIver,C.M. and Monahan,J.J. (1981) *Biochim. Biophys. Acta*, **655**, 243-250.
Prestayko,A.W., Tonato,M. and Busch,H. (1970) *J. Mol. Biol.*, **47**, 505-515.
Price,D.H. and Parker,C.S. (1984) *Cell*, in press.
Reddy,R. and Busch,H. (1983) *Prog. Nucleic Acids Res. Mol. Biol.*, **30**, 127-162.
Reveillaud,I., Lelay-Taha,M.N., Sri-Widada,J., Brunel,C. and Jeanteur,P. (1984) *Mol. Cell. Biol.*, **4**, 1890-1899.
Ricciardi,R.P., Miller,J.S. and Roberts,B.E. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4927-4931.
Rinke,J., Appel,B., Blöcker,H., Frank,R. and Lührmann,R. (1984) *Nucleic Acids Res.*, **12**, 4111-4126.
Rogers,J. and Wall,R.C. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 1877-1879.
Sippel,A.E. (1983) *Eur. J. Biochem.*, **37**, 31-40.
Stunnenberg,H.G. and Birnstiel,M.L. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 6201-6204.
Thimmappaya,B., Weinberger,C., Schneider,R.J. and Shenk,T. (1982) *Cell*, **31**, 543-551.
Tinoco,I., Borer,P.N., Dengler,B., Levine,M.D., Uhlenbeck,O.C., Crothers, D.M. and Gralle,J. (1973) *Nature New Biol.*, **246**, 40-41.
Uhlenbeck,O.C., Carey,J., Romaniuk,P.J., Lowary P.T. and Beckett,D. (1983) *J. Biomol. Structure Dynam.*, **1**, 539-552.
Walter,P. and Blobel,G. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 7112-7116.
Walter,P. and Blobel,G. (1982) *Nature*, **299**, 691-698.
Weaver,R.F. and Weissmann,C. (1979) *Nucleic Acids Res.*, **7**, 1175-1193.
Yang,V.W., Lerner,M.R., Steitz,J.A. and Flint,S.J. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 1371-1375.
Zeller,R., Nyffenegger,Th. and De Robertis,E. (1983) *Cell*, **32**, 425-434.
Zinn,K., DiMaio,D., Maniatis,T. (1983) *Cell*, **34**, 865-879.

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