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Structural and Functional Characterization of Mouse U7 Small Nuclear RNA Active in 3′ Processing of Histone Pre-mRNA

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Oligonucleotides derived from the spacer element of the histone RNA 3′ processing signal were used to characterize mouse U7 small nuclear RNA (snRNA), i.e., the snRNA component active in 3′ processing of histone pre-mRNA. Under RNase H conditions, such oligonucleotides inhibited the processing reaction, indicating the formation of a DNA-RNA hybrid with a functional ribonucleoprotein component. Moreover, these oligonucleotides hybridized to a single nuclear RNA species of approximately 65 nucleotides. The sequence of this RNA was determined by primer extension experiments and was found to bear several structural similarities with sea urchin U7 snRNA. The comparison of mouse and sea urchin U7 snRNA structures yields some further insight into the mechanism of histone RNA 3′ processing.

The specific 3′ termini of histone mRNAs of higher eucaryotes arise by posttranscriptional processing (3, 4, 16) in a reaction dependent on small nuclear ribonucleoproteins (snRNPs) of the Sm serotype (9, 26, 27) and on an additional heat-labile activity (9a, 17). In sea urchins, the rare U7 small nuclear RNA (snRNA) was identified as the active RNA component, based on a biochemical complementation assay in *Xenopus laevis* oocytes (26–28). This assay also permitted the demonstration, by compensatory mutations, of base-pairing interactions between U7 snRNA and the distal portion of the bipartite RNA processing signal present in histone pre-mRNA (20). The primary sequence of this distal spacer element is conserved among higher eucaryotic histone genes (4, 13, 31), albeit with some notable differences between sea urchins and vertebrates (4, 31). The functional importance of the spacer element for histone pre-mRNA processing has also been established in mammalian cells (25).

We based our strategy for characterizing the mammalian homolog of sea urchin U7 snRNA on three main considerations: (i) the primary sequences of mammalian and sea urchin U7 snRNAs may differ too much to allow detection by cross-hybridization (G. M. Gilmartin and M. L. Birnstiel, personal communication); (ii) the base-pairing interaction between U7 snRNA and the distal RNA processing signal of histone pre-mRNA, for which there is good experimental evidence in the sea urchin (20), must be similarly important in mammals; and (iii) the mammalian consensus for this distal sequence motif may therefore be sufficiently complementary to U7 snRNA to be used for its detection and characterization. Using synthetic DNA oligonucleotides derived from the consensus sequence of this distal element, we have been able to characterize mouse U7 snRNA both by a functional inhibition assay and by Northern blot hybridization. Moreover, we have used such oligonucleotides to specifically prime the synthesis and determine the nucleotide sequence of U7 cDNA.

MATERIALS AND METHODS

Oligonucleotide synthesis. Oligonucleotides were synthesized on a Pharmacia Gene Assembler. Sometimes mixtures of two or three nucleotides were used for a single elongation step.

RNase H and micrococcal nuclease inactivation of processing extracts. Nuclear extracts competent for histone RNA 3′ processing were prepared from K21 mouse mastocytoma cells as described previously (9, 17). Such extracts were preincubated with synthetic oligonucleotides for 15 min at 15°C in the presence of 2.7 mM $MgCl_2$ (15) and then supplemented with 20 mM EDTA to inactivate the RNase H. Preincubation of extract with micrococcal nuclease was performed as described previously (9a, 14). After such treatments, histone-specific RNA 3′ processing was assayed by incubating the mixtures at 30°C for 90 min with 30,000 cpm of radioactively labeled precursor RNA from plasmid SP65-H4-119/70 (9). The reaction mixtures were extracted and analyzed by polyacrylamide gel electrophoresis as described previously (17).

Preparation and blot hybridization of snRNAs. Total nuclear RNA was isolated from K21 cells (17) and enriched for snRNAs by precipitation with 2 M LiCl. Small lithium-soluble RNAs (see Fig. 2) or RNAs isolated from K21 nuclear extracts (see Fig. 5) were separated by electrophoresis on 15% polyacrylamide gels (7 M urea, 1 mM EDTA, 90 mM Tris borate [pH 8.3]) and transferred to GeneScreen Plus membranes (New England Nuclear Corp.) by electroblotting. Hybridization of the filters with about 10^6 cpm of individual oligonucleotides, 5′ end labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP, per ml was carried out overnight at 30°C in 6× SSC (1× SSC is 0.15 M NaCl–0.015 M sodium citrate), 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone 360 (Sigma Chemical Co.), 0.2% Ficoll 400 (Pharmacia Fine Chemicals), 1% sodium dodecyl sulfate, and 100 μg of *Escherichia coli* carrier DNA per ml. Filters were washed successively in 6× SSC at 0 and 4°C (see Fig. 2) or two times at room temperature (see Fig. 5).

Synthesis and sequence analysis of U7 cDNA. snRNA prepared as described above was subjected to preparative gel electrophoresis on a 15% polyacrylamide gel. To identify the region of interest, a small amount of the same RNA that had previously been 3′ end labeled with [^{32}P]pCp and T4 RNA ligase (7) was run in the same gel lane, and 5′-end-labeled DNA size markers (*Hpa*II digest of pBR322) were run in a parallel lane. A gel slice containing 65-nucleotide-long RNA was excised, and the RNA was recovered by elution, phenol and chloroform extractions, and repeated ethanol precipitations. In some experiments, the RNA was

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polyadenylated with *E. coli* adenylate transferase (a gift of A. Schmid and M. A. Billeter, Institut für Molekularbiologie I der Universität Zürich) and unlabeled ATP (1, 23). Reverse transcriptions with Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Inc.), with either unmodified RNA and 5'-end-labeled U7-specific oligonucleotides as primers or poly(A)$^+$ RNA and oligo(dT) as the primer, were carried out according to the specifications of the manufacturer. Synthesis of second strands complementary to oligo(dT)-primed first-strand cDNA was performed with a 5'-end-labeled U7-specific oligonucleotide as the primer and with the Klenow fragment of DNA polymerase (Boehringer Mannheim Biochemicals). All primer extension products were gel purified and subjected to Maxam-Gilbert sequencing (18) as modified for short oligonucleotides (instruction manual; P-L Biochemicals, Inc., Milwaukee, Wis.).

RESULTS AND DISCUSSION

Consensus oligonucleotides of the histone spacer element interact with an essential RNP component of the histone RNA 3' processing apparatus. In Fig. 1a, we have aligned and compiled the sequences of the conserved spacer elements of 18 higher vertebrate histone genes (of chicken, human, and mouse origin, coding for all five types of histone proteins) (5, 6, 11, 12, 21, 22, 24, 29, 30, 32). In this region, the sequence AARGAGCTGK (R = A or G, K = G or T) is particularly well conserved and thus represents a good candidate for base-pairing interactions with U7 snRNA.

A mixed oligonucleotide corresponding to the consensus sequence of this region (Fig. 1c) was synthesized by using mixtures of two or three nucleotides in some of the elongation steps. This oligonucleotide was tested for a possible interaction with U7 snRNPs in a histone pre-mRNA processing reaction (9, 17). The rationale of the test is that, if the oligonucleotide can base pair with an essential sequence of U7 snRNA, an attack of the resulting DNA-RNA hybrid by RNase H should destroy that RNA sequence and thus greatly reduce the processing activity. This strategy has previously been used to demonstrate essential base-pairing interactions between the 5' end of U1 snRNA and the 5' splice site of mRNA precursors in an in vitro splicing system (15). Preliminary experiments demonstrated that nuclear extracts from K21 mouse mastocytoma cells (17) contain sufficient RNase H activity to guarantee an efficient cleavage of hybrids between an exogenously added RNA and a complementary DNA oligonucleotide (data not shown). We incubated such a nuclear extract with the synthetic consensus oligonucleotide in the presence of 2.7 mM $MgCl_2$ (a necessary condition for RNase H activity [15]) and then supplemented it with 20 mM EDTA to inactivate the RNase H. The histone-specific RNA processing activity, as subsequently measured in an in vitro processing reaction, was reduced about two- to fivefold by this preincubation (Fig. 1b, lane 4). Control preincubations of nuclear extract with Mg^{2+} either alone (lane 1) or in the presence of an unrelated oligonucleotide of similar length (lane 3) did not impair the processing activity, as compared with a preincubation in the presence of 10 mM EDTA (lane 2) or to no preincubation (data not shown). Moreover, a preincubation of the extract with this consensus oligonucleotide in the presence of 10 mM EDTA also had no inverse effect on the processing activity (data not shown; see Fig. 5). Thus it seemed that the consensus oligonucleotide was indeed able to base pair with an essential RNA component of the histone processing apparatus, but that this interaction was of a very transient nature, such that RNase H digestion was necessary for processing to be inhibited.

To improve this interaction, we analyzed a series of variants of the consensus oligonucleotide in the same inhibition assay. These experiments demonstrated that the inhibition of histone RNA 3' processing, at least at one position, was highly dependent upon the sequence of the oligonucleotides. Specifically, oligonucleotide variant 2, containing T or C at position 12, did not inhibit processing (Fig. 1b, lane 5). In contrast, variants 4 and 5, both of which contain a G at

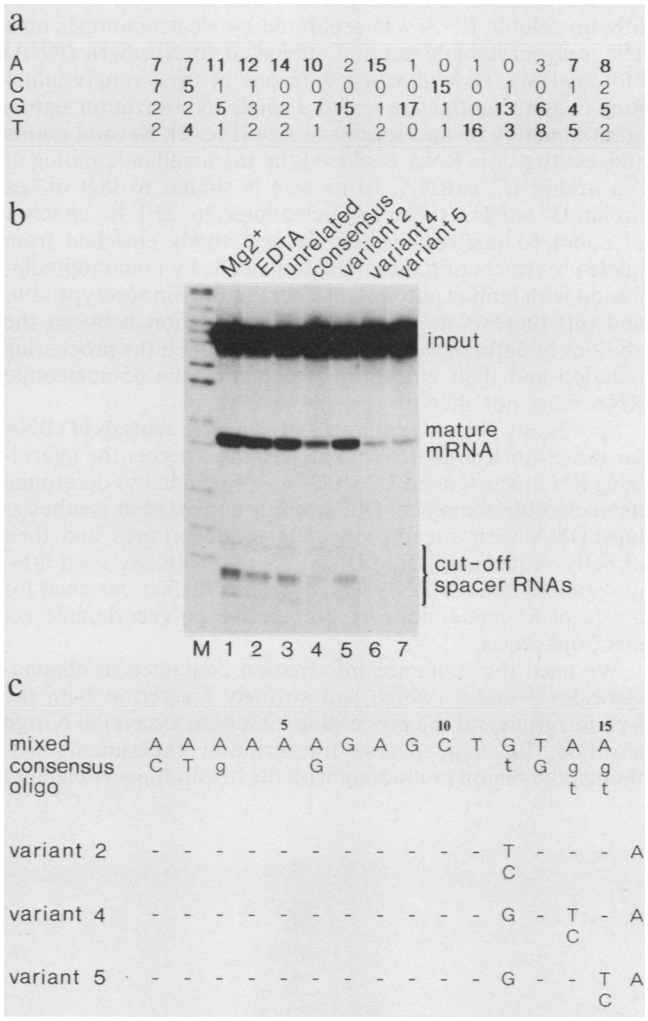

FIG. 1. Inhibition of histone RNA 3' processing by oligonucleotides containing the spacer motif of the conserved processing signal. (a) Sequence compilation of mouse, human, and chicken histone genes in the area of the spacer motif of the RNA 3'-processing signal (5, 6, 11, 12, 21, 22, 24, 29, 30, 32). (b) Oligonucleotide-directed, RNAse H-dependent inhibition of processing. A nuclear extract from K21 mouse mastocytoma cells was preincubated in the presence of the following (lanes): 1, 2.7 mM $MgCl_2$; 2, 10 mM EDTA; 3 through 7, 2.7 mM $MgCl_2$ plus 50 ng of the indicated oligonucleotides. Then 20 mM EDTA was added to inhibit RNase H, and histone-specific RNA 3' processing was measured as described previously (9, 17). M, ^{32}P-end-labeled size marker (*Hpa*II digest of pBR322). (c) Structure of the histone-specific oligonucleotides used in B. Lowercase letters indicate that, during synthesis, these nucleotides were added as a smaller fraction than those written with uppercase letters. Dashes indicate identities of variant oligonucleotides with the original consensus oligonucleotide.

this position (the other alterations at positions 14 and 15, respectively, did not seem to have additional effects), were more efficient inhibitors than the consensus oligonucleotide (lanes 6 and 7). This very strongly suggested that the putative U7 snRNA contains a C at the corresponding position.

Consensus oligonucleotides hybridize to a 65-nucleotide RNA. We next tested whether the oligonucleotides that efficiently interacted with the histone processing component could also be used to detect a corresponding snRNA in a filter hybridization experiment. To this end, we isolated total nuclear RNA from K21 cells (17) and enriched this preparation for snRNAs by a precipitation with 2 M LiCl. The small lithium-soluble RNA was separated by electrophoresis on a 15% polyacrylamide gel and subjected to Northern (RNA) blot analysis. Hybridization with one of the strongly inhibiting oligonucleotides revealed a single hybridization signal, approximately 65 nucleotides in size (Fig. 2). Several points suggest that this RNA is indeed the mammalian homolog of sea urchin U7 snRNA: (i) its size is similar to that of sea urchin U7 snRNA (57 or 58 nucleotides [26, 27]); (ii) an RNA of about 65 nucleotides can be selectively enriched from nuclear extracts of mouse hepatoma cells by immunoprecipitation with human autoimmune sera of the Sm serotype (19); and (iii) there is at least a rough correlation between the abilities of different oligonucleotides to inhibit the processing reaction and their efficiency at detecting the 65-nucleotide RNA (data not shown).

Specifically primed synthesis and sequence analysis of cDNA for the 65-nucleotide RNA. To determine whether the hybridizing RNA was indeed U7 snRNA, we decided to determine its nucleotide sequence. Our strategy consisted in synthesizing cDNA with specific oligonucleotide primers and then directly sequencing that cDNA. As template, we used lithium-soluble nuclear RNA which we had further enriched for a size of 65 nucleotides by preparative polyacrylamide gel electrophoresis.

We used the sequence information contained in oligonucleotides 4 and 5 (which had strongly reacted in both the hybridization and the processing inhibition assays) to design a primer for first reverse transcription experiments. Although the region interacting with the histone spacer element

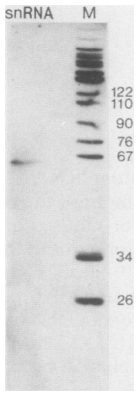

FIG. 2. Northern blot hybridization with mixed oligonucleotides. Nuclear RNA from K21 cells was extracted, and large RNAs were removed by LiCl precipitation. The lithium-soluble RNA was separated by gel electrophoresis, transferred to a GeneScreen filter membrane, and hybridized with ^{32}P-labeled oligonucleotide $\frac{AAA}{CTG}AA\frac{A}{G}GAGCT\frac{GT}{TC}\frac{A}{g}\frac{A}{g}$ A. M, ^{32}P-end-labeled size marker (*Hpa*II digest of pBR322).

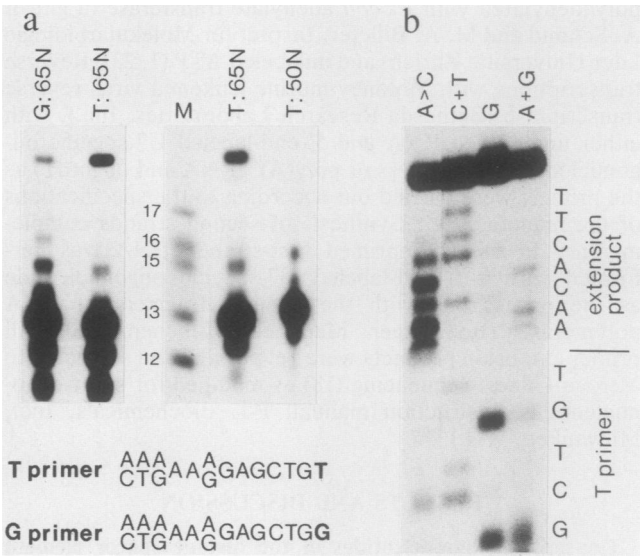

FIG. 3. Primer extension and sequence analysis of 65-nucleotide RNA. (a) A 65-nucleotide fraction (65N) of snRNA from K21 cells was reverse transcribed in the presence of either one of two 5′-end-labeled primer oligonucleotides (T or G). A control reaction was carried out with a fraction of smaller RNA molecules (of approximately 50 nucleotides [50N]), recovered from the same preparative gel as the 65N fraction. The reaction products were separated on a 15% polyacrylamide gel. M, Size markers (mixture of 5′-end-labeled oligonucleotides of the indicated lengths). (b) The major 20-nucleotide extension product was eluted from the gel and sequenced by the Maxam-Gilbert procedure.

lies almost at the 5′ end of U7 snRNA in the sea urchin (20, 27), we were hoping to obtain sequence information for at least a few additional nucleotides up to the 5′ end. Since reverse transcriptase requires a good base pairing at the 3′ end of the primer, we synthesized two 13-mers identical to variants 4 and 5 for the first 12 nucleotides but ending in a 3′-terminal T and G, respectively (Fig. 3a). These primers were 5′ end labeled to a similar specific activity and used in separate but otherwise identical extension reactions. Both reactions yielded one major extension product of about 20 nucleotides (Fig. 3a). The yield of this product was about 5- to 10-fold higher with the primer ending in a 3′-terminal T, indicating that U7 snRNA, or at least the major species, contains A at the corresponding position. A control reaction performed with approximately 50-nucleotide-long RNA recovered from the same preparative gel yielded none of the 20-nucleotide extension product (Fig. 3a, lane T:50N). Considering the specific activity of the primer, the amount of RNA used, and an efficiency of reverse transcription determined to be about 20 to 30% (data not shown), we estimate the template RNA (i.e., U7 snRNA) to be present in about 10^4 copies per cell, but this figure will have to be verified by more precise measurements. The 20-nucleotide extension product was isolated and subjected to Maxam-Gilbert sequencing. The sequence revealed that reverse transcription had resulted in the addition of exactly seven nucleotides to the primer (Fig. 3b).

In the next experiment, we used the newly gathered sequence information to specifically prime the synthesis of the U7 RNA-like strand in a 5′-to-3′ direction. For this purpose, we had to provide the complementary strand as a template. This was obtained by first adding poly(A) tails to the entire 65-nucleotide RNA preparation, by using the

enzyme adenylate transferase from *E. coli*, and then reverse transcribing the tailed RNA with oligo(dT) as a primer. The specific primer for second strand cDNA synthesis, oligonucleotide p+16 (Fig. 4), was complementary to the sequence determined in the previous experiments. However, due to initial sequencing ambiguities, which were subsequently clarified (Fig. 3b), p+16 was lacking the 5'-most nucleotide of the putative U7 sequence and additionally contained mixtures of two different nucleotides at two internal positions (Fig. 4). When this primer was 5' end labeled and incubated with the cDNA template described above in the presence of nucleoside triphosphates and Klenow DNA polymerase, almost all of the extension product was slightly larger than 65 nucleotides and heterogeneous in size, reflecting the variable length of the added poly(A) tail (data not shown). This material was again gel purified and subjected to Maxam-Gilbert sequencing. The extension product was found to contain 44 nucleotides beyond the primer, followed by a run of A's (Fig. 4). Since we cannot rule out that some of these A's were contributed by the original RNA rather than by the added poly(A) tail, we cannot be absolutely certain about the exact 3' end of the putative U7 snRNA.

To determine the sequence across the region previously covered by the primers, we synthesized additional oligonucleotides complementary to more downstream parts of the 65-nucleotide RNA. Oligonucleotides complementary to positions 34 through 49 and 50 through 62 (Fig. 4) yielded little if any U7-specific primer extension product (data not shown), presumably because the corresponding RNA sequence forms part of a large secondary structure (see below). However, the analysis of a full-length extension product obtained with oligonucleotide cB, complementary to positions 18 through 33, confirmed and complemented the sequence information of the 65-nucleotide RNA (Fig. 4). As discussed below, a comparison with sea urchin U7 snRNA and additional functional tests leave no doubt about the identity of this RNA with mouse U7 snRNA. However, because bulk rather than individually cloned cDNA was sequenced and the source of RNA was an established cell line, we cannot rule out some sequence variability contributed by multiple gene copies or by genetic polymorphisms, as has been found for sea urchin U7 snRNA (27). Moreover, as pointed out above, the exact length of U7 RNA cannot be indicated, because of the problem of assigning the first 3'-terminal A's to either U7 RNA itself or to the added poly(A) tail.

Complementary oligonucleotides prove the involvement of the sequenced RNA in histone RNA 3' processing. We argued that, if the determined sequence were really that of mouse U7 snRNA, then the oligonucleotide cA, complementary to the first 16 nucleotides of this RNA (Fig. 4), should be a better inhibitor of histone RNA 3' processing than the previously used mixed oligonucleotides (Fig. 1b). Indeed, oligonucleotide cA completely inhibited histone pre-mRNA processing, regardless of whether the conditions allowed RNase H to cleave any resulting RNA-DNA hybrid (Fig. 5a, lanes 2 and 3). Moreover, half-maximal inhibition was still achieved with as little as 1 ng of cA, whereas 5 to 10 ng of the previously used oligonucleotide variants 4 and 5 had been required to obtain the same effect (data not shown). Thus, the interaction of oligonucleotide cA with the processing factor was considerably more efficient than that of these mixed oligonucleotides with shorter complementarities to U7 snRNA. Moreover, we confirmed the previous finding (9a) that histone RNA 3' processing is sensitive to micrococcal nuclease digestion (Fig. 5a, lane 4), thus independently proving that an RNA component participates in the processing reaction.

To confirm that these various treatments had the expected effects on the 65-nucleotide RNA, we isolated RNA from nuclear extracts pretreated as in Fig. 5A, lanes 1 to 4, and subjected them to polyacrylamide gel electrophoresis and Northern blot analysis. This blot was hybridized with a mixture of end-labeled complementary oligonucleotides covering the entire length of the sequenced RNA. The hybridizing RNA had become shortened, albeit to a different extent, after an incubation of the nuclear extract with either micrococcal nuclease (Fig. 5b, lane 4) or oligonucleotide cA under RNase H conditions (2.7 mM $MgCl_2$; lane 2). In contrast, no shortening was observed when the extract had been preincubated with cA in the presence of 10 mM EDTA (Fig. 5b, lane 3). Therefore the inhibition seen in the processing reaction (Fig. 5a, lane 3) must have been due to some very stable base-pairing interaction and not to a residual

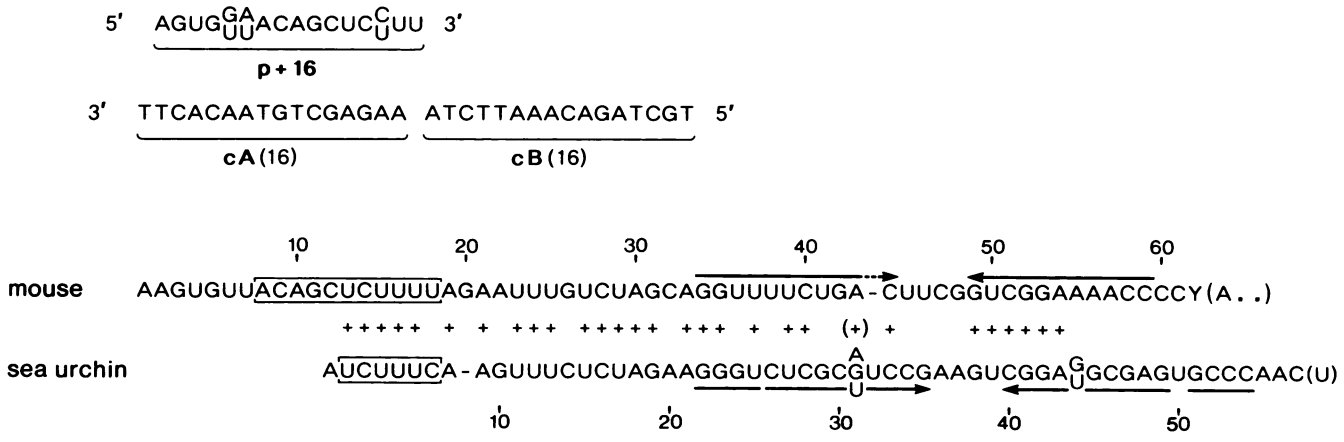

FIG. 4. Comparison of the nucleotide sequences of mouse and sea urchin U7 snRNAs. The sequence of mouse U7 snRNA was determined by Maxam-Gilbert sequencing of primer extension products as specified in the text. Dashes indicate gaps that were introduced to optimize the alignment with the sequence of sea urchin U7 snRNA (27). Nucleotide identities between the two sequences are indicated by plusses, regions of dyad symmetry are indicated by arrows, and the regions complementary to the histone spacer element are indicated by boxes. A 3'-terminal oligo(A) tail added in one experiment is indicated in brackets, since the first of these A's might belong to U7 snRNA. Shown above are the sequences of several oligonucleotides used in primer extensions and other experiments as specified in the text.

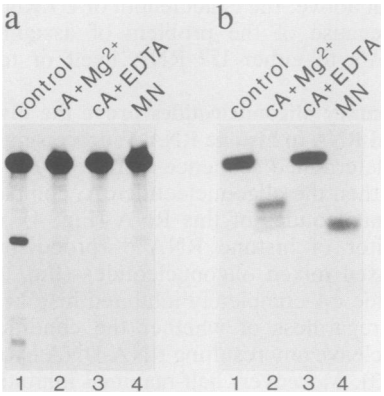

FIG. 5. (a) Inhibition of histone RNA 3′ processing by oligonucleotide cA complementary to the 5′ end of mouse U7 snRNA. Nuclear extract from K21 cells was preincubated in the presence of the following (lanes): 1, 10 mM EDTA; 2 and 3, 50 ng of oligonucleotide cA (Fig. 4) plus 2.7 mM $MgCl_2$ (lane 2) or 10 mM EDTA (lane 3); 4, micrococcal nuclease. Otherwise, the experimental conditions were as described in the legend of Fig. 1. (b) Specific cleavage of U7 snRNA by RNase H in the presence of complementary oligonucleotide cA or by micrococcal nuclease. RNAs from extracts preincubated as in (a) were separated by gel electrophoresis and transferred to a GeneScreen filter membrane as described in the legend to Fig. 2. The filter was hybridized with a mixture of ^{32}P-labeled oligonucleotides complementary to the entire length of U7 snRNA. Each lane contained approximately 2×10^7 cell equivalents of RNA.

RNase H activity. These experiments functionally confirm the identity of the sequenced RNA with U7 snRNA active in histone pre-mRNA 3′ processing. Moreover, it is evident that a large portion of the 5′ end of this RNA is freely accessible, be it to a hybridizing nucleic acid molecule or to an attacking nuclease.

Comparison of the nucleotide sequences of mouse and sea urchin U7 snRNAs reveals important structural and functional features. The identification of the sequenced RNA as U7 snRNA also becomes evident when one compares it with sea urchin U7 snRNA (27) (Fig. 4). The two RNAs are almost colinear over a large part of the sequence. Depending on which sea urchin U7 variant is taken as the reference, 28 or 29 nucleotides are identical in the two RNAs. However, these identical nucleotides are interspersed with diverging ones, which might explain why a vertebrate U7 snRNA has not been previously detected by cross-hybridization. Both RNAs contain a long hairpin loop structure near the 3′ end, but there are differences in its primary sequence, and the stem of the mouse RNA is devoid of the bulges present in the sea urchin. The sequence complementary to the histone spacer element is similarly located near the 5′ end in both species. In contrast, mouse U7 RNA is lacking an additional complementarity to the proximal part of the histone processing signal present in the sea urchin (27) but whose functional significance is unclear (F. Schaufele, Ph.D. thesis, Universität Zürich, 1986). A region required for the assembly of sea urchin U7 snRNA into Sm-precipitable snRNPs has been localized between positions 9 and 20 (10). Of these 11 nucleotides, 9 are identical in the mouse sequence.

An interesting difference is that the sequence complementarity to the histone spacer element is longer in the mouse than in the sea urchin and is located at a greater distance from the hairpin-loop structure. Similarly, the histone spacer element differs between sea urchins and vertebrates with respect to both its sequence and its distance from the proximal dyad symmetry element (4, 31). However, when each U7 sequence is aligned with one of its target histone mRNA precursors (sea urchin H3 and mouse H4, respectively), we note that the hairpin loops and complementary regions of the U7 and histone RNAs can be aligned perfectly in the mouse and almost so in the sea urchin (Fig. 6). In other words, the distance between the palindromic and base-pairing elements is practically the same for U7 and histone RNA within one species. From this, we speculate that an alignment of the two RNAs via a juxtaposition of these structural elements on either side of the cleavage site may be critical for RNA processing to occur. Data in agreement with this model are that the sequences but not the secondary structures of the histone (2; Schaufele, Ph.D. thesis) and U7 palindromes (10) may be significantly altered without greatly affecting the efficiency of processing. Moreover, any attempt to alter the distance of the two elements in sea urchin H3 pre-mRNA resulted in a complete loss of processing activity (8), and sea urchin U7 snRNA tolerated the loss of maximally two or the gain of maximally four nucleotides between

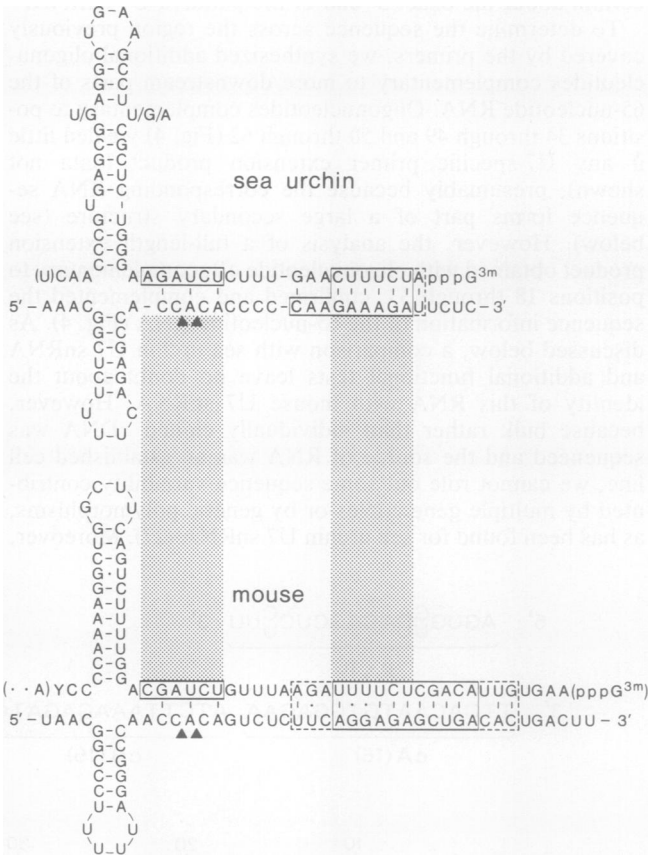

FIG. 6. Model for the interaction of U7 snRNA with histone pre-mRNA during RNA 3′ processing. The sequences of sea urchin and mouse U7 snRNAs are aligned with two of their target histone pre-mRNAs, sea urchin (*Psammechinus miliaris*) H3 and mouse H4, respectively. Note the similar or identical distance between the regions of base complementarity in the spacer element (boxes and shaded area on the right-hand side) and the two stem-and-loop structures of U7 and histone RNAs. In both species, the palindromic U7 sequence UCUAGN (boxes and shaded area on the left-hand side) lies opposite the histone sequence ACCACA that contains the major cleavage sites (triangles). ^{3m}Gppp indicates the 5′-terminal trimethyl guanasine cap structure demonstrated only for sea urchin U7 snRNA (26).

these two regions (10). However, it must be pointed out that those U7 spacing mutants that were defective for histone RNA processing had also lost their ability to become incorporated into Sm-precipitable snRNPs (10). Further mutational analyses of this kind would therefore be necessary to either prove or disprove the above model. According to this model, the palindromic U7 hexanucleotide UCUAGN becomes positioned opposite the histone sequence ACCACA, which spans the 3′ end of the mature mRNA (9). The alignment of these two sequences might form the target for a site-specific endonuclease that would cleave the histone pre-mRNA.

The length difference between mouse and sea urchin U7 snRNAs is mostly due to differences at the 5′ end, and one may ask why this is so. It has been noted that the histone spacer element is more loosely conserved in vertebrates than in sea urchins (4, 31), where it almost invariably takes on the form CAAGAAAGA. The extra 5′ sequence of mouse U7 snRNA could therefore substitute for insufficient base pairing with individual histone pre-mRNAs within the spacer element proper. Consistent with this notion, our experiments with micrococcal nuclease and with the complementary oligonucleotide cA indicated that a large portion of the 5′ end of mouse U7 snRNA is freely accessible (Fig. 5), whereas a treatment of sea urchin nuclear extracts with micrococcal nuclease removed only about eight nucleotides from the 5′ end of U7 snRNA (10). Interestingly, the mouse H4 gene differs from the conserved AARGAGCTGK sequence at three positions (GGAGAGCTGA), but additional base pairs could be formed both 5′ and 3′ to this sequence (Fig. 6). Thus, the vertebrate histone RNA 3′ processing system may have acquired a greater sequence flexibility than is found in the sea urchins.

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