Identification of the Human U7 snRNP as One of Several Factors Involved in the 3′ End Maturation of Histone Premessenger RNA's

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In eukaryotic cells, the conversion of gene transcripts into messenger RNA's involves multiple factors, including the highly abundant small nuclear ribonucleoprotein (snRNP) complexes that mediate the splicing reaction. Separable factors are also required for the 3′ end processing of histone pre-mRNA's. The two conserved signals flanking the 3′ cleavage site are recognized by discrete components present in active HeLa cell extracts: the upstream stem loop associates with a nuclease-insensitive factor, while binding to the downstream element is mediated by a component having the properties of a snRNP. The sequence of the RNA moiety of the low abundance human U7 snRNP suggests how the relatively degenerate downstream element of mammalian pre-mRNA's could be recognized by RNA base-pairing.

THE 3′ ENDS OF MOST EUKARYOTIC RNA'S ARE FORMED BY RNA processing rather than transcription termination (*1*). The majority of cellular messenger RNA's (mRNA) become polyadenylated after cleavage of a longer precursor (*2*), whereas the 3′ end of histone mRNA's are generated by simple RNA cleavage (*3, 4*). Flanking the site of 3′ end formation in histone pre-mRNA's are two conserved sequence elements that appear to be required for maturation (*3, 5*); these are a hairpin loop structure immediately upstream and a purine-rich region 13 to 17 nucleotides (nt) downstream of the site of cleavage (*6, 7*). In the well-studied sea urchin histone genes (*3, 5*), both elements are highly conserved, the downstream element always having the sequence CAAGAAGA (*6*). By contrast in vertebrate species (*7*), the downstream element is quite variable, although a consensus of $^{A}_{G}$AAAGAGCTG can be derived for mammalian genes.

Roles in RNA processing have recently been defined for a number of small nuclear ribonucleoprotein (snRNP) particles. snRNP's of the Sm class each contain (at least) one U-RNA with its characteristic trimethylguanosine (m_3G) 5′ cap complexed with one or more proteins reactive with anti-Sm, a group of autoantibodies found in the sera of patients with various rheumatic diseases (*8–10*). The most abundant Sm snRNP's (about 10^6 per cell)—the U1, U2, U5, and U4/U6 particles—are involved in pre-mRNA splicing (*11*). In addition, a number of low-abundance Sm snRNP's exist. Their RNA components include the U7 snRNA from sea urchin (*12*), the mammalian U8-U10 snRNA's (*13*), and other less well-characterized species in various eukaryotes (*14*). An as yet unidentified snRNP has been implicated in polyadenylation (*15*).

The 57-nt sea urchin U7 snRNA, which is about one-thirtieth as abundant as U1 RNA (*12*), is required for proper 3′ processing of sea urchin histone H3 transcripts in the *Xenopus* oocyte (*16*) and has been proposed to interact with both conserved sequence elements in the histone pre-mRNA (*12*). An experiment where a processing defect in a sea urchin H3 gene was suppressed by introducing compensatory base changes into U7 snRNA (*17*) provided strong support for a base-pairing interaction with the downstream element.

In mammalian systems, much less is known about histone mRNA 3′ end processing, and the mammalian counterpart of U7 snRNA has eluded identification. Studies in in vitro systems that generate correct 3′ termini on mammalian histone transcripts (*18, 19*) have established that the cleavage is endonucleolytic (*18*) and requires Sm snRNP's (*18, 19*). Processing appears to be cell-cycle regulated (*20*), most likely involving a heat-labile non-snRNP factor (*21*). Ribonuclease (RNase) protection experiments have revealed two distinct interactions of the pre-mRNA with factors containing Sm determinants (*19*). On addition of the substrate to the reaction mixture, protected fragments containing the upstream conserved hairpin loop are recovered; later in the reaction, the protection extends to include the cleavage site and the downstream purine-rich sequence element.

Here we have identified and characterized two separable factors that are required for mammalian histone pre-mRNA 3′ processing in vitro; one of these is a snRNP. Binding and protection experiments indicate that a non-snRNP factor binds the upstream hairpin loop element, while downstream binding is mediated by a snRNP. To elucidate how the snRNA in this particle might form an RNA-RNA hybrid with the degenerate downstream sequence element in mammalian histone gene transcripts, we have identified and sequenced the human U7 snRNA.

Treatment with micrococcal nuclease inhibits histone pre-mRNA 3′ processing in vitro. RNA processing reactions that require snRNP components should be sensitive to prior treatment of the active nuclear extract with micrococcal nuclease, which can be inactivated by chelating essential Ca^{2+} ions. Synthetic mouse histone H3 precursor RNA was incubated in the presence of untreated nuclear extract (Fig. 1, lane 1); the 248-nt precursor gives rise to a previously characterized product RNA of about 200 nt (*19*). Prior incubation of the extract at 30°C in the presence of micrococcal nuclease and Ca^{2+} efficiently degrades the small RNA's in the extract and abolishes the appearance of product (Fig. 1, lane 5). Control reactions show that prior incubation alone (Fig. 1, lane 2), the presence of Ca^{2+} ions (Fig. 1, lane 3), or the addition of micrococcal

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nuclease in the presence of EGTA (to chelate Ca^{2+}) (Fig. 1, lane 4) has no effect on the processing activity. We conclude that a nuclease-sensitive factor, most probably a snRNP, is required for this mammalian processing reaction in vitro.

At least two factors are required for processing activity. Fractionation studies of HeLa nuclear extracts have allowed the characterization of multiple components required for the splicing of premessenger RNA's. Analysis of three fractions obtained by gel filtration on a Bio-Gel A1.5m column (*22*) shows that more than one component is also required for histone 3′ processing activity (Fig. 1B). Pooled fraction A (lane 2) contains structures of large molecular size ($>10^6$ daltons), including most snRNP's; pooled fraction B (lane 3) contains some U1 snRNP's and proteins ranging between 100 and 500 kD; and pooled fraction C (lane 4) contains only proteins of less than 100 kD (*22*). None of these fractions alone exhibits significant processing activity relative to the unfractionated extract (lane 1). However, fractions A and B combined produce a good yield of product (lane 5), while combinations of fractions A and C (lane 6) or B and C (lane 7) do not regenerate activity. Moreover, the activity of A plus B plus C is no greater than A plus B alone.

We next tested these fractions for their ability to complement a micrococcal nuclease-treated extract (Fig. 1C). As expected, mixing the inactive nuclease-treated extract with untreated extract restores activity, showing that nuclease treatment does not generate an inhibitor of the reaction. Fraction A (Fig. 1C, lane 3) likewise restores significant activity, while addition of either fraction B or C (lanes 4 and 5) or addition of RNA alone (*Escherichia coli* 16*S*) to the reaction (lane 6) does not. We conclude that fraction A contains the nucleic acid factor or factors required for 3′ processing activity and that a different active component or components (possibly a protein) is present in fraction B.

Separable components mediate recognition of the two conserved sequence elements. Previous RNase protection experiments revealed two different interactions between histone pre-mRNA's and factors containing Sm determinants (*19*) (summarized in Fig. 2A). Protected RNA fragments recovered with anti-Sm from the human H3 RNA substrate at time zero (Fig. 2B, lane 2) include the upstream conserved hairpin loop structure, fragment b. After 30 minutes, two additional major RNA fragments, a and f, are immunoprecipitated (lane 3); fragment a extends the region of early protection to include the downstream conserved sequence element, whereas fragment f represents the protection of the capped 5′ end of the transcript (*19*) and (Fig. 2A).

When protection experiments were performed with nuclear extract inactivated by prior treatment with micrococcal nuclease (Fig. 2B, lanes 4 to 6), protection of the downstream sequence element (fragment a) at 30 minutes was abolished, suggesting that the Sm-containing component interacting with the downstream region is a snRNP. In contrast, fragment b, representing protection of the hairpin loop, is recovered both at time zero (lane 4) and 30 minutes of reaction (lane 5), indicating that the anti-Sm–reactive factor binding upstream of the cut site is insensitive to nuclease.

We further probed the interaction of the required reaction components with the pre-mRNA substrate by RNase protection experiments with fractionated nuclear extract. Both the zero and 30-minute profiles of the control unfractionated extract (Fig. 2C, lanes 1 to 3) can be reconstituted (as can the processing activity; see Fig. 1B) when fractions A and B are combined (lanes 4 to 6). As expected, none of the fractions alone produced a normal binding profile (Fig. 2D). Instead, fraction A (lanes 1 and 2) gave hairpin loop protection (fragment b), which increased with time but only to a low level. Fraction B gave a high level of hairpin loop protection (fragment b) at both times (Fig. 2D, lanes 4 and 5), but no downstream protection was observed even after long exposures of lane 5. No protection at all was detected with fraction C (lanes 7 to 9). Fraction B thus appears to contain a factor or factors capable of binding independently to the hairpin loop, whereas A (the snRNP fraction) is required, in addition, for significant interaction with the downstream element.

Processing activity is abolished by oligonucleotide-targeted RNase H treatment. Inactivation of a specific snRNP in a complex extract by deoxyoligonucleotide-directed RNA cleavage with RNase H has effectively demonstrated a requirement for U1, U2, and U4/

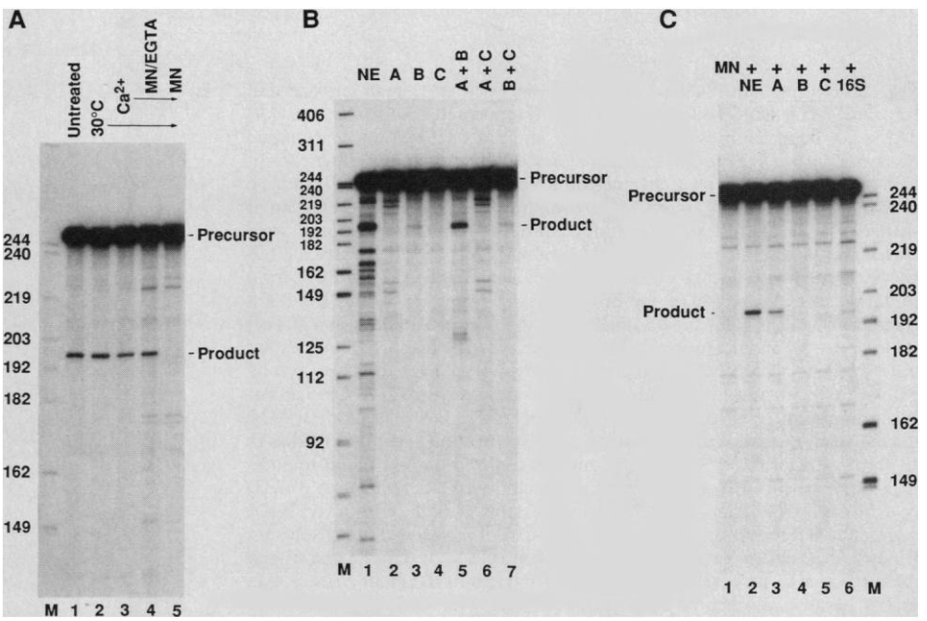


Fig. 1. In vitro 3′ end processing of mouse histone H3 pre-mRNA with nuclease-treated and fractionated nuclear extracts. (**A**) HeLa nuclear extract (*32*) was first treated (lane 5) with micrococcal nuclease (MN; Cooper Biomedical) at 5000 U/ml in 1 m*M* $CaCl_2$, then 4m*M* EGTA was added. Control samples were: (lane 1) no prior incubation; (lane 2) prior incubation for 30 minutes at 30°C; (lane 3) with 1 m*M* $CaCl_2$; (lane 4) micrococcal nuclease with 1 m*M* $CaCl_2$ and 4 m*M* EGTA. Synthetic mouse histone H3 precursor RNA (*19*) was labeled with [α-^{32}P]GTP (guanosine triphosphate) (60,000 cpm) and incubated with nuclear extract for 30 minutes under processing conditions (*19*) in 5 m*M* EDTA. RNA isolated from the processing reactions (*19*) was resolved on a 6 percent sequencing gel. The 248-nt precursor and 200-nt product RNA's are indicated at the right; the sizes (in nucleotides) of DNA markers (lane M) are at the left. (**B**) Nuclear extract (*33*) was fractionated over Bio-Gel A-1.5m (Bio-Rad) in buffer D with 15 m*M* $MgCl_2$ (*22*). Pooled, dialyzed fractions A to C were provided by V. Gerke. The in vitro processing reactions (in 10 m*M* EDTA) and RNA analyses were as in (A). (Lane 1) The reaction products obtained with unfractionated nuclear extract (NE); (lane 2) fraction A; (lane 3) fraction B; (lane 4) fraction C; (lane 5) combinations of fractions A and B; (lane 6) A and C; (lane 7) B and C. (**C**) Nuclear extract was inactivated by micrococcal nuclease (MN) treatment (lane 1) and used for in vitro processing [as in (A)] after supplementation with nuclear extract (NE) (lane 2); with pooled fractions prepared as in (B), fraction A (lane 3), fraction B (lane 4), or fraction C (lane 5); or with 1 μg of *E. coli* 16*S* ribosomal RNA (lane 6).

U6 snRNP's in the mammalian pre-mRNA splicing reaction (*23, 24*). To identify a snRNP involved in the mammalian histone 3′ processing reaction, we synthesized three oligonucleotides with potential complementarity to mammalian U7 RNA (Fig. 3A). We designed these oligomers to mimic the downstream sequence element required for histone mRNA processing since this element seems to interact directly with a snRNP factor based on our own observations (Fig. 2B) and work in the sea urchin system (*17*).

We first asked whether 3′ processing activity was affected by treatment of the nuclear extract with these oligonucleotides and RNase H (Fig. 3A). The untreated extract (lane 1) shows the mouse H3 248-nucleotide precursor and its 200-nt product. In contrast, the product band does not appear in extract samples first treated with RNase H and oligonucleotides C15 (lane 7), containing the mammalian consensus sequence for the downstream element, or with oligonucleotide M314 (lane 9), containing the downstream sequence of the mouse H3 gene. When oligonucleotides C15 (lane 2) or M314 (lane 3) were added without RNase H and Mg^{2+}, the processing was undiminished, demonstrating that they do not act simply as competitors of the reaction. Prior incubation of the nuclear extract with Mg^{2+} alone (lane 4) also had no effect on activity. Prior incubation with Mg^{2+} and RNase H in the absence of an oligonucleotide gave reduced but clearly detectable activity (lane 5). The level was the same after prior treatment with oligonucleotide 5′C (lane 6), complementary to the 5′ end of U1 snRNA [formerly called Ula, as in (*24*)], or somewhat surprisingly with H315 (lane 8), which contains the downstream sequence of a human H3 gene. Thus, two of the three oligonucleotides designed to corresond to histone pre-mRNA sequences target a factor present in the nuclear extract required for histone pre-mRNA 3′ processing in vitro. This factor is very likely the mammalian U7 snRNP.

A 60-nt m_3G-capped Sm RNA is targeted by oligonucleotide-directed RNase H digestion. We next used our histone-derived oligonucleotides and RNase H to search for the RNA component of the human U7 snRNP. Since it had been established that Sm snRNP's are involved in mammalian histone 3′ end processing (*18, 19*), we analyzed RNA's obtained by serial immunoprecipitation with anti-Sm and anti-m_3G (*25*). This procedure yields a highly purified preparation (Fig. 3B, lane 1) containing the major Sm RNA's—U1, U2, U4, and U5 (which have m_3G 5′ cap structures)—as well as other less abundant species. A doublet band of about 60 to 65 nt (indicated by arrows) is markedly reduced in intensity when the sample is first treated with RNase H and either of the histone-derived oligonucleotides, C15 (lane 3) or M314 (lane 5). By contrast, this doublet is unaffected by treatment with RNase H and oligonucleotide 5′C, which degrades only U1 snRNA (lane 2), or by treatment with oligonucleotide H315 (lane 4). The degradation of the 60-nt RNA mirrored the effect of each oligonucleotide on the histone pre-mRNA processing activity: C15 and M314 caused both loss of activity and disappearance of the band, while H315 did not (Fig. 3A). Thus, a candidate mammalian U7 snRNA is contained in an Sm snRNP and has a m_3G-5′ cap structure. It is quite scarce; in the 3′ end-labeled preparation analyzed (Fig. 3B), the relative intensity of the doublet band in lane 3 is less than one-fiftieth that of U4 snRNA.

The sequence of human U7 snRNA shows complementarity to the histone downstream element. The 3′ end of the candidate U7 snRNA (Fig. 3B) was analyzed by partial digestion of the 3′ end-labeled RNA with a set of base-specific ribonucleases (*26*). The 3′ prime end sequence of the major (upper) band was read as

5′-CACCNGAAAGCCCCN-OH

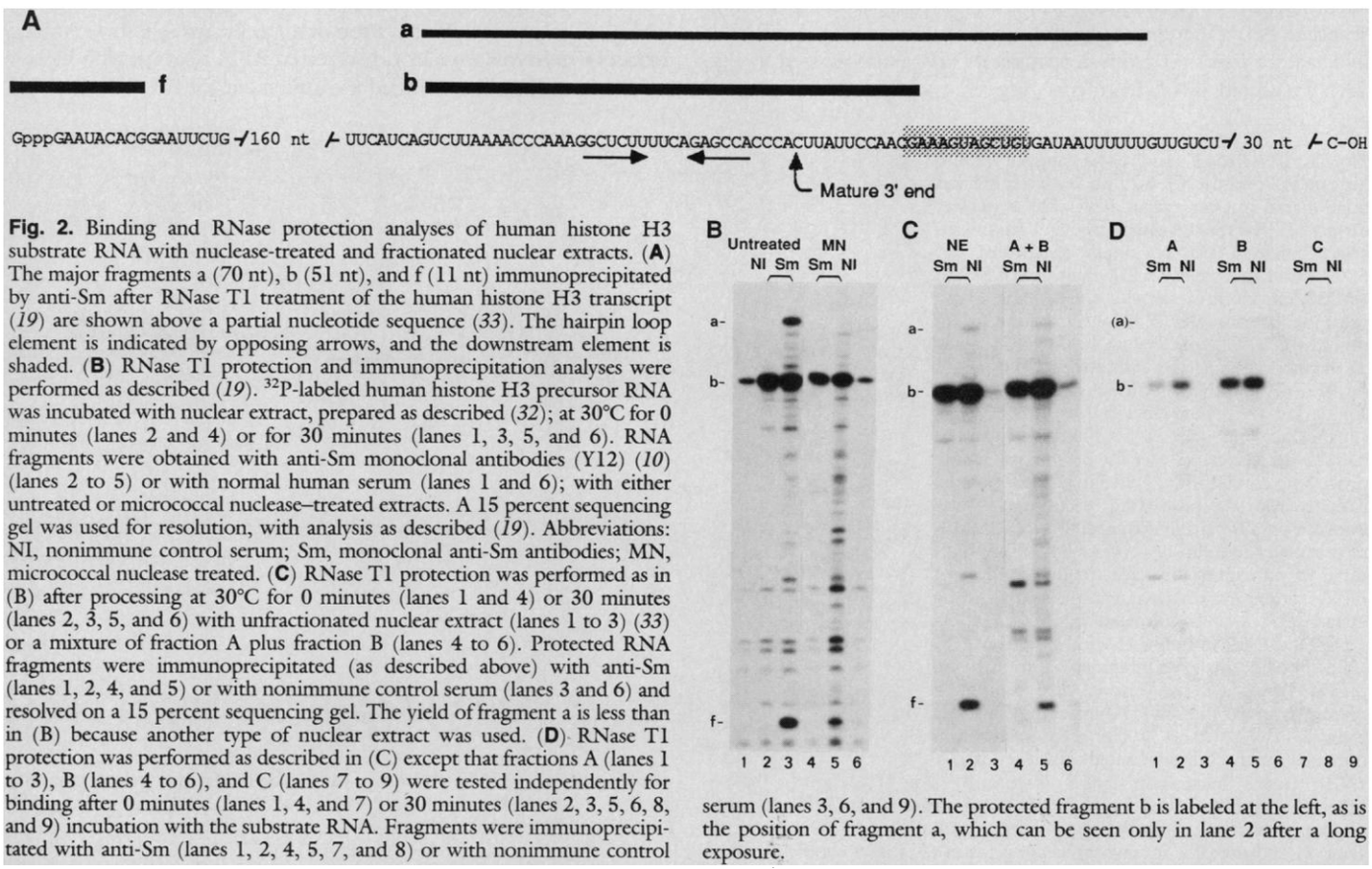


Fig. 2. Binding and RNase protection analyses of human histone H3 substrate RNA with nuclease-treated and fractionated nuclear extracts. (**A**) The major fragments a (70 nt), b (51 nt), and f (11 nt) immunoprecipitated by anti-Sm after RNase T1 treatment of the human histone H3 transcript (*19*) are shown above a partial nucleotide sequence (*33*). The hairpin loop element is indicated by opposing arrows, and the downstream element is shaded. (**B**) RNase T1 protection and immunoprecipitation analyses were performed as described (*19*). ^{32}P-labeled human histone H3 precursor RNA was incubated with nuclear extract, prepared as described (*32*); at 30°C for 0 minutes (lanes 2 and 4) or for 30 minutes (lanes 1, 3, 5, and 6). RNA fragments were obtained with anti-Sm monoclonal antibodies (Y12) (*10*) (lanes 2 to 5) or with normal human serum (lanes 1 and 6); with either untreated or micrococcal nuclease–treated extracts. A 15 percent sequencing gel was used for resolution, with analysis as described (*19*). Abbreviations: NI, nonimmune control serum; Sm, monoclonal anti-Sm antibodies; MN, micrococcal nuclease treated. (**C**) RNase T1 protection was performed as in (B) after processing at 30°C for 0 minutes (lanes 1 and 4) or 30 minutes (lanes 2, 3, 5, and 6) with unfractionated nuclear extract (lanes 1 to 3) (*33*) or a mixture of fraction A plus fraction B (lanes 4 to 6). Protected RNA fragments were immunoprecipitated (as described above) with anti-Sm (lanes 1, 2, 4, and 5) or with nonimmune control serum (lanes 3 and 6) and resolved on a 15 percent sequencing gel. The yield of fragment a is less than in (B) because another type of nuclear extract was used. (**D**) RNase T1 protection was performed as described in (C) except that fractions A (lanes 1 to 3), B (lanes 4 to 6), and C (lanes 7 to 9) were tested independently for binding after 0 minutes (lanes 1, 4, and 7) or 30 minutes (lanes 2, 3, 5, 6, 8, and 9) incubation with the substrate RNA. Fragments were immunoprecipitated with anti-Sm (lanes 1, 2, 4, 5, 7, and 8) or with nonimmune control serum (lanes 3, 6, and 9). The protected fragment b is labeled at the left, as is the position of fragment a, which can be seen only in lane 2 after a long exposure.

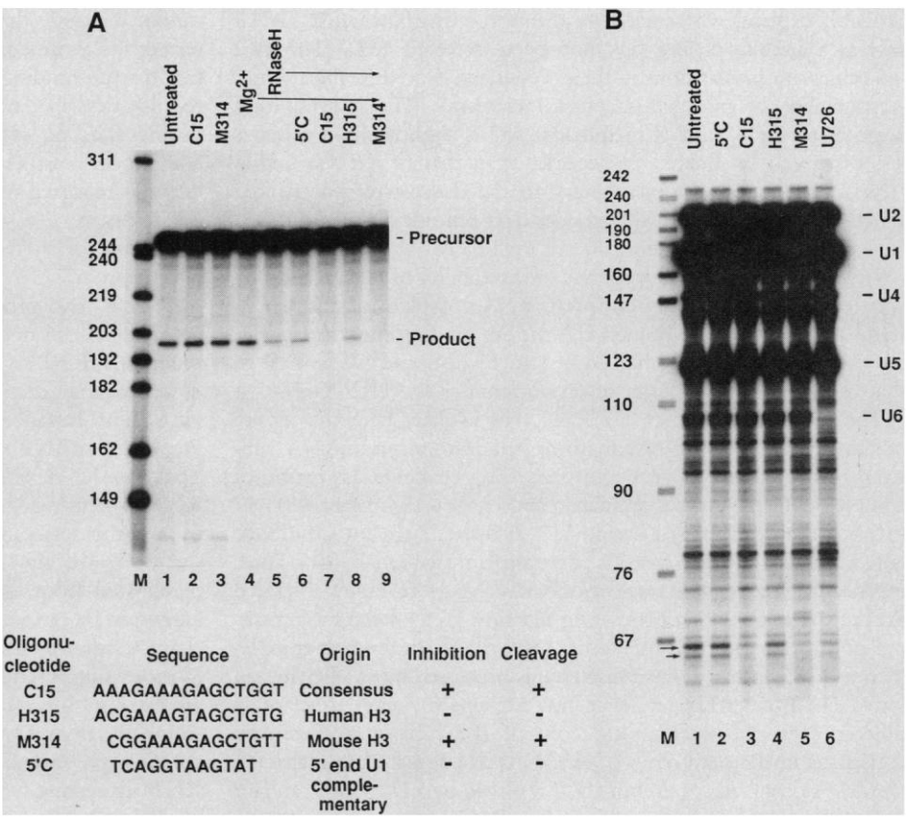
Limited analysis of the lower band indicated a related sequence possibly shorter at its 3′ end. The 3′ terminal N base was determined to be a U by thin-layer chromatography of a T2 RNase digest of the RNA.

A set of four 5′ end-labeled oligonucleotides complementary to the probable 3′ end sequence, each containing a different base at the position corresponding to the internal N position in the above sequence were used to prime the synthesis of complementary DNA (cDNA). The substrate used for reverse transcription was total RNA extracted from an anti-Sm precipitate of a sonicated preparation of HeLa cells (*27*). A primer extension product of about 65 nt was seen most strongly with the oligonucleotide that contained C, less strongly when a T was present, and not at all with either G or A. We conclude that the base at the internal N position of U7 snRNA is probably a G since the oligonucleotide providing a G·C base pair was most efficient in priming synthesis by reverse transcriptase and the oligonucleotide giving a G·T base pair primed to some extent.

We next used the 5′ end-labeled oligonucleotide U726 (5′-AGGGGCTTTCCGGTG-3′) to prime the synthesis of a full-length single-stranded cDNA. The complementarity of this oligonucleotide to the 3′ end of the candidate U7 snRNA was confirmed by RNase H cleavage (Fig. 3B, lane 6). The cDNA was eluted from a single band on a polyacrylamide gel and its sequence was determined by chemical cleavage (*28*). The sequence derived is unconfirmed because (i) no complementary strand was available for DNA sequence analysis and (ii) there are inherent ambiguities in enzymatic RNA sequencing. However, the sequence of the primer-extended product could be read unambiguously and can be considered a good representation until a clone of a true human U7 RNA gene can be analyzed.

Near the 5′ end of the sequence of human U7 snRNA is a region of perfect complementarity (CAGCUCUUUU) to the mammalian histone pre-mRNA downstream consensus sequence (ÅAAAGAG-CUG) (Fig. 4). No obvious complementarity is found to the other conserved sequence element, the hairpin loop. The 3′ end of human U7 RNA can be folded to form a strong hairpin, which leaves both the histone pre-mRNA complement and a sequence (AUUUG) believed to direct the binding of Sm antigens (*29*) available for interaction with other molecules. The U7 sequence also explains the relative abilities of the three deoxyoligonucleotides used in our RNase H experiments to abolish in vitro processing activity (Fig. 3A) or to cleave U7 RNA (Fig. 3B). The C15 (consensus) and M314 oligonucleotides are complementary to U7 over ten contiguous positions, whereas the H315 sequence has an extra nucleotide that would create a bulge in the middle of the DNA-RNA helix.

Role of the U7 snRNP in histone pre-mRNA processing. We have identified and sequenced the RNA component of the first minor snRNP of known function in mammalian cells. The human U7 snRNP, which is required for correct 3′ processing of histone pre-mRNA's, is closely related to the snRNP's involved in splicing in that it contains proteins with Sm antigenic determinants and a 5′ m_3G cap on its RNA moiety. Its abundance appears to be less than 1/200 that of the U1 snRNP (based on pCp labeling) (Fig. 4, legend). This low copy number (about 10^3 per cell), however, is not incompatible with the estimates that histone mRNA's comprise about 1 percent of the total message and that an average mammalian pre-mRNA contains ten introns. Perhaps a higher relative abundance of the U7 snRNP is required in sea urchin eggs (1/30 of U1) (*12*) because of the rapid rate of DNA replication that will occur after fertilization.



Oligonucleotide	Sequence	Origin	Inhibition	Cleavage
C15	AAAGAAAGAGCTGGT	Consensus	+	+
H315	ACGAAAGTAGCTGTG	Human H3	-	-
M314	CGGAAAGAGCTGTT	Mouse H3	+	+
5'C	TCAGGTAAGTAT	5' end U1 complementary	-	-

Fig. 3. In vitro 3′ end processing of mouse histone H3 pre-mRNA after oligonucleotide-directed RNase H treatment. (**A**) HeLa nuclear extract (*32*) was first treated with RNase H and deoxyoligonucleotides at 40 μg/ml (*24*). After addition of EDTA to 10 m*M* and mouse ^{32}P-labeled H3 precursor RNA, in vitro processing reactions were carried out and analyzed on a 6 percent sequencing gel as described (Fig. 1A). (Lane 1) Reaction products obtained with untreated nuclear extract alone; (lane 2) in the presence of oligonucleotide C15; (lane 3) M314; (lane 4) nuclear extract that had been first incubated with 5 m*M* $MgCl_2$ alone; (lane 5) with RNase H and 5 m*M* $MgCl_2$; (lane 6) with RNase H and $MgCl_2$ plus oligonucleotide 5′C; (lane 7) C 15; (lane 8) H315; or (lane 9) M314. The chart lists the sequence of each oligonucleotide, its origin (*7, 34, 35*), and its effect on both processing and cleavage of the 60- to 65-nt target RNA. The mouse H3 precursor and product RNA's are indicated at the right and the sizes of the DNA markers are labeled at the left in nucleotides. (**B**) Sm snRNP's were immunoprecipitated (*27*) from a HeLa whole cell extract with monoclonal anti-Sm. Extracted RNA's were labeled at their 3′ ends with T4 RNA ligase and [5′-^{32}P]pCp (*36*), immunoprecipitated with anti-m_3G (*25*), treated with RNase H and deoxyoligonucleotide at 40 μg/ml as above, and fractionated on a 15 percent urea sequencing gel. (Lane 1) Profile of Sm RNA's in the untreated sample; (lane 2) treated with RNase H and the 5′C (U1) oligonucleotide; (lane 3) oligonucleotide C15; (lane 4) H315; (lane 5) M314; (lane 6) U726. Arrows at the lower left indicate bands affected by treatment with histone-derived oligonucleotides. A lighter exposure of lane 2 shows that U1 RNA is degraded by treatment with oligonucleotide 5′C. The degradation of U6 RNA (lane 6) is due to a six-base complementarity between the primer oligonucleotide U726 and U6 RNA. The abundant m_3G-capped Sm RNA's are labeled at the right and the sizes of DNA markers (in nucleotides) are given at the left. The relative intensities of the end-labeled RNA bands may be due in part to different efficiencies of the 3′ end residues as acceptors for pCp (*36*).

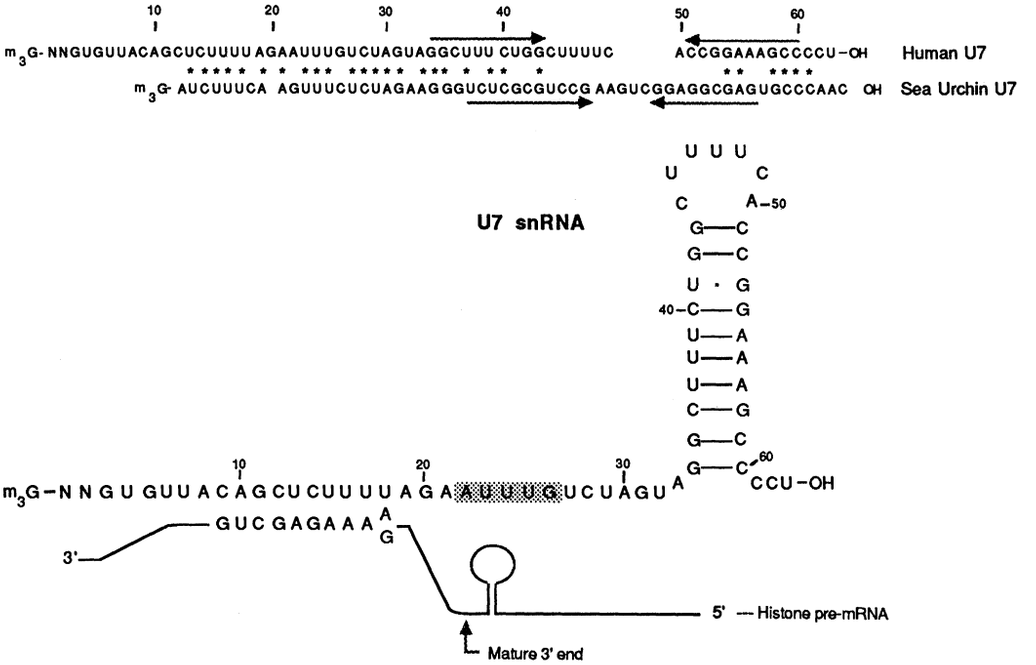


Fig. 4. Sequence of human U7 snRNA. The major 3′ end-labeled candidate U7 RNA was excised from a 15 percent gel such as that shown in Fig. 3B, and its sequence was analyzed enzymatically (*26*) with base-specific ribonucleases (Pharmacia) T_1, U_2, Phy M, B-cereus, and C13. The 3′ end nucleotide was determined by thin-layer chromatography (*37*) of a complete digest of the end-labeled RNA. Oligonucleotide U726 (AGGGGC-TTTCCGGTG), complementary to the sequence at the 3′ end of the RNA, was labeled at its 5′ end with [γ-^{32}P]ATP (adenosine triphosphate) and T4 polynucleotide kinase and used to prime synthesis by reverse transcriptase in reactions (*38*) typically containing 60 μg of primer and 150 μg of template Sm RNA. The full-length cDNA of 60 to 65 nucleotides was isolated from a single band on an 8 percent sequencing gel and subjected to chemical sequence analysis (*28*). The resulting sequence of human U7 snRNA (*39*) is compared above to that of sea urchin U7 (*30*); identical nucleotides are indicated. Below, a possible secondary structure for human U7 RNA with the region complementary to the histone pre-mRNA downstream signal shown by its potential base pairing interaction. The putative Sm binding sites (*29*) are shaded.

In addition to their similar sizes, the nucleotide sequences of human U7 (63 nt) and sea urchin U7 (57 nt) (*12, 30*) reveal considerable homology (Fig. 4). There is about 50 percent overall sequence identity, but no contiguous stretch longer than 5 nt. This probably explains why cross-hybridization of mammalian U7 RNA with sea urchin probes has not been detected (*31*). The two molecules are similar both in their secondary structures and in their arrangement of putative functional elements. The hairpin loop adjacent to the 3′ end of mammalian U7 is slightly shorter, but is less disrupted by bulged nucleotides than that of the sea urchin RNA. The region complementary to the downstream conserved signal in histone pre-mRNA's resides near but not directly at the 5′ end in human U7 as it does in sea urchin. An "Sm binding site" (*29*), which would account for the precipitability of both RNA's by anti-Sm, appears between the pre-mRNA complement and the 3′ stem-loop structure. In human U7, the Sm binding site conforms to the consensus (AU_nG, where n = 3 to 6) (*29*) and is identical to that of mammalian U1 over seven nucleotides (AAUUUGU).

The sequence of human U7 RNA may explain the ability of the mammalian processing system to accept histone pre-mRNA substrates with variant downstream processing elements. Its region of complementarity to the mammalian consensus is significantly longer (10 bp) than that of sea urchin U7 (6 bp) (*12*) to the absolutely conserved sea urchin histone downstream sequence (*6*). Thus, mismatches or bulged nucleotides may be more easily tolerated during the mammalian processing reaction. Yet, of the four mammalian histone pre-mRNA's tested (*19*), that with the best complementarity to human U7 (mouse H3) is processed most efficiently in vitro. The human U7 sequence also explains the single nucleotide difference in the overlapping core of the histone downstream consensus in the sea urchin (GAAAGA) as compared to mammals ($\overset{A}{G}$AAAGA): a C in sea urchin U7 is replaced by a U (which can pair with either A or G) in the mammalian RNA.

Base-pairing with the U7 snRNP is not the only interaction occurring during the 3′ end maturation of mammalian histone pre-mRNA's. Our fractionation studies indicate that a nuclease-insensitive factor recognizes the upstream conserved hairpin loop structure. Binding occurs very rapidly in vitro and may serve to promote the subsequent snRNP interaction with downstream sequences. The crude fraction (B) that contains this binding factor is required in addition to the snRNP fraction (A) for processing activity, but we cannot conclude that both binding and complementation are conferred by the same molecule or molecules. It is also unclear whether our hairpin-binding factor might be the same component as the recently described heat-sensitive factor (*21*) whose activity fluctuates during the cell cycle (*20*). We find that the upstream binding component is associated with an Sm antigen, whereas the heat-labile factor is reported not to be immunoprecipitated by antisera of the Sm serotype.

The use of snRNP's which engage in base-pairing interactions with a pre-mRNA substrate, is a feature common to histone pre-mRNA 3′ end processing and the splicing reaction occurring in mammalian cell nuclei (*1, 11*). Since only one rather than several different snRNP's (*11*) are apparently involved, we expect that the 3′ processing reaction will prove more amenable to dissection in vitro. For instance, now that the human U7 RNA has been identified, snRNP reconstitution studies can be easily undertaken. The two RNA processing systems have also evolved to include RNA-protein recognition events. It is most intriguing that binding of both the upstream hairpin in histone pre-mRNA's (our data) and the 3′ splice site (*22*) are carried out by factors that can be dissociated from snRNP's yet have Sm determinants—otherwise known to be carried only by integral snRNP proteins (*27*). Further characterization of non-snRNP components involved in the histone 3′ processing reaction promises to clarify not only how many factors are essential but also how activity is coupled to the cell cycle. The simplicity of our system offers advantages over the much more complex pre-mRNA splicing reaction for the study of regulated RNA processing.

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"No, I'm not a ghost, Mr. Scrooge. But I'm one highly agitated hologram."