Different complexes are formed on the 3' end of histone mRNA with nuclear and polyribosomal proteins

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ABSTRACT

Specific protein-RNA complexes are formed by incubating a synthetic histone mRNA 3' end (a 30 nucleotide stem-loop structure) RNA with extracts of either nuclei or polyribosomes. The complex formed between the stem-loop and nuclear proteins has a lower electrophoretic mobility than the complex formed between the stem-loop and polyribosomal proteins. Binding of the synthetic 3' end by both polyribosomal and nuclear proteins is abolished when two of the conserved uridine residues in the loop are replaced with adenosines. UV crosslinking of the protein complexes to the synthetic RNA resulted in transferring radiolabel to similar sized proteins, 50 kD, in both the nuclear and polyribosomal extracts.

INTRODUCTION

Histone genes lack introns and their mRNAs are not polyadenylated. Instead the histone mRNAs end in a highly conserved stem loop sequence, consisting of a 6-nucleotide stem and 4 nucleotide loop (1,2). The 3' end of the histone mRNA is formed by an RNA processing reaction and this is the only processing reaction prior to transport of the histone mRNA to the cytoplasm. Formation of the 3' end requires recognition of the stem-loop as well as recognition of a purine rich sequence which occurs about 10–15 nts 3' of the stem loop by the U7 snRNP, followed by cleavage just 3' of the stem loop sequence (3–5). The stem-loop sequence at the 3' end of the histone mRNA has multiple functions; it is responsible for regulating the half-life of histone mRNAs in the cytoplasm (6,7), as well as being involved in processing the pre-mRNA (8,9).

Sequences in mRNA which are involved in regulating mRNA metabolism presumably carry out their role by binding to specific proteins. In the case of the transferrin receptor mRNA, a protein has been identified which interacts with a specific stem-loop sequence in the 3' untranslated region and regulates the half-life of the transferrin receptor mRNA (10–13). The same protein interacts with a similar structure in the ferritin mRNA and regulates the translation of the ferritin mRNA (10–13).

We report here the presence of proteins found both in the nucleus and the polyribosomes which specifically bind the stem loop structure of histone mRNA. The resulting complexes are different as judged by the differing mobility of the two complexes in a non-denaturing polyacrylamide gel. However SDS-PAGE analysis of the proteins after UV-crosslinking to the stem-loop RNA showed that the polysomal and nuclear proteins each has molecular weights of approximately 50 kD.

MATERIALS AND METHODS

Preparation of nuclear extract

Mouse myeloma cells were grown in suspension culture and harvested at a concentration of $4-6 \times 10^5$ per ml, while they were in exponential growth. Nuclear extract was prepared as described by Mowry and Steitz (4). The extracts (10 mg/ml protein) were dialyzed against 0.08M KCl, 10% glycerol, 10 mM HEPES, pH 7.5 and stored in liquid N₂.

Polyribosome preparation

Polyribosomes were prepared from mouse myeloma cells exponentially growing by modification of the method of Ross and coworkers (14). Cells were collected by centrifugation and suspended in cold RSB (10 mM KCl, 10 mM Tris·HCl, pH 7.5, 2 mM Mg(OAc)₂) buffer at a concentration of 1×10^8 cells per ml. After swelling the cells for 15 minutes, they were broken by homogenization with 10–20 strokes of a B pestle in a Dounce homogenizer. The homogenate was centrifuged at $1000 \times g$ for 2 min to remove the nuclei. The supernatant was collected and centrifuged again at $8,000 \times g$ for another 10 min. The supernatant from this centrifugation was adjusted to 0.1% Triton X-100 and layered on a 30% sucrose pad containing $1 \times$ RSB and centrifuged at $100,000 \times g$ for 3 hours.

The polysomal pellet was washed gently with cold RSB twice to remove traces of sucrose. The polyribosomes were suspended with one or two strokes in a 2 ml Teflon-glass homogenizer in cold storage buffer (Tris·HCl 25mM, pH 7.5, 1mM DTT, 50mM KCl and 50% glycerol). They were then stored at -70°C in 100 μl aliquots.

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Salt extraction of polysomes

The polyribosomes were thawed on ice and 2M KCl was added so that the final KCl concentration was 0.5M or 0.8M. Similar results were obtained with both salt concentrations. We obtained a higher yield of binding activity with a 0.8M extract, but the properties of the binding activity were similar. The mixture was incubated on ice with gentle shaking for 30 minutes. The mixture was then centrifuged for 4 hours at $100,000\times g$ to remove the ribosomes. The supernatant was collected carefully and dialyzed against 1 liter of cold buffer containing 25mM Tris, pH7.5, 50mM KCl, 1mM DTT, and 20% glycerol overnight. A small amount of precipitated material was removed by centrifugation and discarded. The supernatant was stored in 100 μ l aliquots at -70°C .

Preparation of the radiolabeled stem-loop RNA substrate

Two single stranded oligonucleotides, having 18 complementary bases which form the T7 RNA polymerase promoter were annealed. The longer one has 30 additional bases that form an overhang which the T7 RNA polymerase uses as a template to synthesize the stem-loop RNA as described by Uhlenbeck and coworkers (15). The reaction was carried out in a 50 μ l volume in 40mM TrisHCl, pH 8.0, 6mM MgCl_2 , 5mM DTT, and 1mM spermidine, 200 μ M of the three unlabeled ribotriphosphates and 100 μ Ci of the fourth ribotriphosphate (5 μ M, specific activity = 400 Ci/mM). The sequences of the two oligonucleotides are shown below:

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3' ATTATGCTGAGTGATATCCGGGTTTTCGAGAAAAGTCTCGGTGGGT 5'
5' TAATACGACTCACTATAG                                     3'
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The radiolabeled products were then separated on a 20% preparative gel and the full-length product eluted from the gel. The RNA was precipitated with ethanol in the presence of 20 μ g of yeast tRNA, collected by centrifugation and dissolved in 1 mM EDTA. The sequence of the wild-type RNA used as a probe is shown in Fig. 1. Additional RNA substrates were synthesized using oligonucleotides with an identical sequence except for the indicated changes in the stem-loop sequence.

For some experiments a synthetic RNA was synthesized on an Applied Biosystems oligonucleotide synthesizer using RNA phosphoramidites from Peninsula Laboratories. The synthetic RNA was end-labeled with polynucleotide kinase using ^{32}P -ATP. Identical results were obtained with both RNA probes in the mobility shift experiments (data not shown).

The longer probe used in Fig. 5 was synthesized from a clone in pGEM3 containing the histone H2a-614 3' end (extending from 8 nts before the stem-loop to 35 nts past the histone 3' end) or from the same clone which has been digested with *Hinf*I (which cuts 10 nucleotides past the histone 3' end, just before the U7 snRNP binding site) and then recloned into pGEM3. Riboprobe transcripts of equal length were synthesized with T7 RNA polymerase from each of these clones after digestion with different enzymes in the remaining polylinker 3' of the histone 3' end.

Mobility shift experiments

20,000 cpm (10 femtomoles) of radiolabeled RNA was dried down together with yeast tRNA (10–100 μ g) in a microfuge tube. The reaction was routinely carried out in a 20 μ l volume. The final reaction routinely contained 10mM Tris pH7.5, 50 mM KCl, 1mM DTT, 10% glycerol and 5–30 μ g of protein. The mixture was incubated on ice for 20 minutes to allow binding

before being loaded onto a nondenaturing 6% polyacrylamide gel (ratio of acrylamide:bisacrylamide was 40:1 in a buffer containing 6.7mM Tris, pH7.5, 3.3mM NaOAc, pH 5 and 1mM EDTA) without any dye. Tracking dye was loaded onto a separate lane. Electrophoresis was continued until the bromophenol blue marker reached the bottom of the gel and the complexes were visualized by autoradiography. Competition experiments were performed with unlabeled stem-loop RNA that was synthesized on the DNA synthesizer or by using T7 RNA polymerase.

For the experiments using the longer substrates (Fig. 4) the conditions were modified to include 50 μ g/ml heparin (16). After incubation on ice for 20 min, the complexes were treated with 500 μ g/ml RNase A for 5 min at 0°C and then loaded onto a 4% polyacrylamide non-denaturing gel.

UV-crosslinking experiments

The binding reaction was carried out as described above and the mixture was aliquoted onto microfuge tube caps. The mixture was irradiated by UV light for 10 minutes at a distance of 3 inches using a 15 watt Sylvania germicidal bulb on ice as described by Greenberg (17,18). The caps were put back on to the tubes and

Figure 1. Structure of the 3' end of histone mRNA. A. The consensus sequence of the histone stem-loop and its presumed secondary structure is shown. The N's represent nucleotides which vary among different histone mRNAs. The sequence of the specific (SL_{wt}) and non-specific probe (SL_{mut}) are also shown. The 2A's in the SL_{mut} probe which differ from the SL_{wt} RNA which have been circled. B. Product of the transcription reaction. The products of the transcription reaction were analyzed by gel electrophoresis. The gel-purified product is shown in lane 1 and the crude product is shown in lane 2. The major products at the bottom of lane 2 (arrow) are the 10-12 nt transcripts which terminated at the base of the stem.

the tubes were centrifuged briefly. RNase A was added to a concentration of 1 mg/ml and incubated at 37°C for 15 minutes. The samples were boiled for 5 min. in 1% SDS, .01M β -mercaptoethanol and loaded onto a 12.5% SDS-PAGE gel. Non-radioactive molecular weight markers were also run simultaneously. The markers were visualized by staining with Coomassie blue and the crosslinked protein was visualized by autoradiography.

RESULTS

Specific binding activities in the nucleus and polysomes

The stem-loop at the 3' end of histone mRNA has been very highly conserved among various animal species. The general structure of the stem loop is shown in figure 1A, drawn as a six base stem and a four base loop. This structure is found in histone RNAs from all vertebrates, as well as echinoderms (19), cnidaria (20), nematodes (21), annelids (22), *Drosophila* (19) and *Volvox* (23). The two GC base pairs at the base of the stem and the UA base pair at the top of the stem are conserved in all these species. Following the two G's at the base of the stem there are usually three pyrimidines, although in some of the genes in annelids and the genes in *Volvox* there is one purine and two pyrimidines. The loop always has a uridine in the third position and has a uridine at the first position in all species except nematodes where there is a C. The second position is usually a uridine also, although it is a cytosine in at least one mouse histone gene, and an A in some sea urchin histone mRNAs (19). The fourth base in the loop varies. There are usually three A's preceding the stem and 4 C's or A's after the stem, at the end of the mature histone mRNA. Thus this structure has been highly conserved among

a large variety of metazoans. In contrast, the histone mRNAs of plants, yeast, fungi, and protists are polyadenylated and lack this stem-loop structure.

To detect proteins which might interact with the 3' end of histone mRNA we synthesized a 30 nucleotide RNA with the sequence of the 3' end of the H2a-614 mRNA (24) using the method of Uhlenbeck and coworkers (15). The sequence of the synthetic RNA (SL_{wt}) is also shown in Figure 1A. As a control for specificity of binding we also synthesized an analog which had 2 A's replacing the two U's in the loop to give the SL_{MUT} RNA. Figure 1B (lane 2) shows the product of the T7 RNA polymerase transcription. In addition to the full-length 30-mer, there is also a major product which has terminated at the base of the stem at the start of the stem-loop. The 30-mer purified by elution from the gel (Fig. 1B, lane 1) was used as a probe in the subsequent experiments.

The synthetic stem-loop was incubated with proteins extracted from nuclei or from polyribosomes. The protein-RNA complexes were analyzed by gel electrophoresis under non-denaturing conditions. Figure 2 shows the results of the mobility-shift assays. Yeast transfer RNA was included as a non-specific competitor, since without tRNA non-specific complexes were formed and most of the probe was found at the top of the gel (Fig. 2A, lane 1). Complexes were formed when the radiolabeled synthetic RNA containing the stem-loop were incubated with either proteins extracted from polyribosomes (Fig. 2A, lane 2) or nuclei (Fig. 2A, lane 4). The complex formed with the polyribosomal extract had a higher mobility than the complex formed with the nuclear proteins. Excess unlabeled stem-loop RNA competed for binding with the labeled probe in both the nuclear and the polysomal extract (Fig. 2A, lanes 3 and 5), indicating that the complex is specific.

Figure 2. Nuclear and polysomal proteins bind the stem-loop. A. 10 femtomoles of radiolabeled stem-loop RNA was mixed with either polysomal (lanes 1,2,3) or nuclear extract (lanes 4,5) and incubated as described in Materials and Methods prior to electrophoresis on a 6% polyacrylamide gel (acrylamide:bisacrylamide = 40:1). Lane 1 shows binding in the absence of tRNA. All other lanes show binding in the presence of 30 micrograms of tRNA as nonspecific competitor. Lanes 2 and 4 show binding in the absence of any cold stem-loop whereas lanes 3 and 5 show binding in the presence of 100 fold excess of unlabeled stem-loop as a specific competitor. N and P indicate the position of the nuclear and polysomal complex respectively. B. Binding of the stem-loop RNA by increasing amounts of polysomal protein is shown. Lanes 1-4 show binding in the presence of 37.5mM KCl and lanes 5-6 show binding in 200mM KCl. The amounts of proteins used were: lane 1, 0µg, lane 2, 15 µg, lanes 3 and 5, 21 µg, lanes 4 and 6, 42 µg. All of the binding assays were done in the presence of 30 µg of tRNA. C. Binding assays similar to the ones shown above were done with nuclear protein with increasing amounts of cold stem-loop RNA as a specific competitor. The first lane shows where the RNA runs. The second lane shows binding in the absence of tRNA and cold competitor. The rest of the lanes show binding with a constant amount of protein (32µg) and 15µg of tRNA. The amount of competitor used in the assays increases from 0 in lane 3 to 1000 times the amount of radiolabeled stem-loop in lane 7. N indicates the position of the specific complex and RNA indicates the input RNA. D. The binding of a SL_{wt} and SL_{mut} probe by nuclear and polysomal protein was compared. The polysomal proteins used (lanes 2 and 4) were from a 0.8 M salt extraction of polysomes. The sequence of the RNAs used is shown in Fig. 1A. Lanes 1 and 2 show binding of the wildtype stem-loop by nuclear and polysomal proteins respectively. Lanes 3 and 4 show binding of a mutant stem-loop by nuclear and polysomal protein respectively. 30µg of tRNA and 40 µg of protein was used throughout. N and P indicate the position of the nuclear and polysomal complex respectively.

Figure 2B shows binding between a constant amount of synthetic stem-loop RNA and increasing amounts (5–70 μ gms) of proteins extracted from polyribosomes. Increasing amounts of complex formed as the amount of protein was increased (Fig. 2B, lanes 1–4) and the amount of complex formed was the same at 37.5 mM and 200 mM KCl (Fig. 2B, lanes 5,6). A similar independence of salt concentration on binding was observed for the nuclear extract (data not shown).

Figure 2C shows binding between nuclear proteins and a radiolabeled stem-loop using constant amounts of protein and tRNA and increasing amounts of cold stem-loop RNA. In the absence of tRNA all the probe was found in a non-specific complex at the top of the gel. In this nuclear extract there were non-specific complexes formed which were not competed by the homologous RNA, as well as a specific complex (N), which was competed by the specific RNA (Fig. 2C, lanes 3–7). Non-specific complexes were observed with some preparations of nuclear or polyribosomal extracts, but specific complexes were always observed.

To demonstrate the specificity of binding the extracts were incubated with probes which lacked the stem-loop sequence or had altered stem-loop sequences. Replacement of the 16nt stem-loop sequence while leaving the flanking sequence the same abolished binding (not shown) indicating that the interaction was with the stem-loop and not the flanking sequence. A similar result was obtained when two substitutions were introduced into the loop sequence. Figure 2D shows that when the U's in the first and third bases of the loop were replaced by A's, we could no longer detect binding of the proteins in both the polyribosomal and nuclear extract (Fig. 2D, lanes 3 and 4), while incubation of the same amount of protein with the same amount of synthetic histone stem-loop resulted in formation of large amounts of complex (Fig. 2D, lanes 1 and 2). In this experiment the polyribosomes were extracted with 0.8M KCl rather than 0.5M

KCl, and a small amount of a complex with a slower mobility was present. Extraction of polyribosomes with 0.8M KCl resulted in a increased yield of the binding activity, although the properties of the binding activity extracted with 0.5M and 0.8M KCl were identical.

Figure 3 shows the effect of small changes in the stem-loop sequence on the specificity of binding of the nuclear and polyribosomal activities. Substrates containing a C-G base pair at the top of the stem or an A in the third base of the loop were synthesized. These two substrates have altered the conserved features of the histone stem-loop sequence. Each of these substrates was incubated with the polyribosomal (Fig. 3A) or nuclear (Fig. 3B) extracts, in parallel reactions with the wild-type substrate. Changing the conserved UA base-pair at the top of the stem to a CG base-pair reduced the complex formation by 80–90%, although there was still residual binding which was proportional to the amount of protein added. A similar reduction in binding affinity was seen observed when the conserved third base of the loop was changed from a U to a A. Again, both the nuclear and the polyribosomal extracts showed a 80–90% reduction in complex formation compared to the wild-type substrate (Figs. 3A and B, lanes 8 and 9). As shown above (Fig. 2D) changing the two conserved bases in the loop resulted in an inability to detect complex formation. Thus the interactions between the stem-loop and the binding activities are extremely sequence specific for the loop region.

A 50kD protein(s) can be crosslinked at the stem-loop with UV light

The complexes formed between the polysomal proteins and the stem-loop and between the nuclear proteins and the stem-loop differ in size. To determine the sizes of the proteins involved in binding, we incubated the radiolabeled stem-loop RNA with extracts from the nucleus or the polyribosomes and then irradiated

Figure 3. Specificity of binding to the stem-loop. A. Synthetic substrates with the wild-type stem-loop sequence (lanes 1–3), with a C-G base-pair replacing the A-U base-pair at the top of the stem (lanes 4–6) or with an A replacing the U in the third base of the loop (lanes 7–9) were incubated in buffer (lanes 1,4 and 7) or with 10 (lanes 2,5,8) or 20 (lanes 3,6,9) μ gm of polysomal protein extract with 0.8M KCl and analyzed as described in Figure 2A. The position of the complex (P) is indicated. B. The same synthetic substrates were incubated in buffer (lanes 1,4 and 7) or with 20 (lanes 2,5,8) or 40 (lanes 3,6,9) μ gm of nuclear protein and analyzed as described in panel A. The position of the complex (N) is indicated.

the reaction with UV light to covalently link the RNA and the protein(s) binding to it. Figure 4A shows an experiment in which the radiolabel used in the synthesis of the substrates was UTP. The RNA was incubated with an extract from polyribosomes, cross-linked with UV light (17) and then treated with RNase prior to electrophoresis. Lane 1 is a control to show the mobility of RNA alone which was treated with UV light. Lanes 2 and 3 have the wild type stem-loop crosslinked in the absence and presence of excess homologous competitor stem-loop respectively. There is a doublet of about 50kD clearly visible in lane 2 which is not present in lane 3. There was no binding observed if the samples were not irradiated (not shown).

Figure 4B shows a similar experiment in which the synthetic RNA was labeled with CTP and incubated with both the polyribosomal and nuclear extracts. These samples were not treated with RNase prior to electrophoresis. Two different probes were used, the normal histone stem-loop (lanes 3 and 4) and the RNA with A's substituted for the U's at position 1 and 3 of the loop (Fig. 4B, lanes 1 and 2). Nuclear extract was used in lanes 1 and 3, and polysomal extract was used in lanes 2 and 4. Crosslinking was only observed when the proteins were incubated with the wild type stem-loop. The protein(s) from the nucleus and the cytoplasm which are crosslinked to the RNA both migrate as doublets of the same size, which is again about 50kD.

The stem-loop binding protein binds to an internal stem-loop sequence independent of the presence of a U7 snRNP binding site

One cytoplasmic function of the stem-loop sequence, regulation of histone mRNA half-life, requires that the stem-loop sequence be present at the 3' end of the mRNA and not internal in the 3' untranslated region (6,25). In the nucleus the histone pre-mRNA resulting from transcription of the histone gene contains the stem-loop internally. This pre-mRNA is bound by a factor which is required for efficient processing and which must bind the stem-loop when it is internal in the RNA precursor (8,9,26).

Figure 4. UV-crosslinking of a 50kD protein to the stem-loop. A. The radiolabeled SL_{wt} probe (labeled with UTP) was incubated with a polyribosomal extract after which they were crosslinked by UV light. The samples in lanes 2 and 3 were treated with RNase before being analyzed on a 12.5% SDS-PAGE. Lane 1 shows RNA crosslinked in the absence of protein and not treated with RNase. Lane 2 shows the crosslinking of wildtype stem-loop to polysomal protein. Lane 3 is identical to lane 2 except that the binding and crosslinking was done in the presence of 100-fold excess of unlabeled SL_{wt} as competitor. The position of the molecular weight markers is indicated. B. The radiolabeled SL_{wt} and SL_{mut} probes (labeled with CTP) were incubated with either 40 μ g of protein extracted from nuclei (lanes 1 and 3) or 40 μ g of protein extracted from polyribosomes (lanes 2 and 4) and then irradiated with UV light. The samples were not treated with RNase before being run on the gel. Lanes 1 and 2 are samples incubated with the SL_{mut} RNA and lanes 3 and 4 are samples incubated with the SL_{wt} RNA. The position of the molecular weight markers is indicated.

The U7 snRNP also binds the pre-mRNA, recognizing a purine-rich sequence 10–20 nts 3' of the stem-loop (4,5). Thus one possible function of nuclear stem-loop binding activities is in RNA processing. Alternatively there could be a nuclear protein which binds to the stem-loop following processing and becomes part of the histone mRNP. Thus the protein which recognizes the stem-loop in the processing reaction would be replaced by a second protein which binds the terminal stem-loop in the mature message.

To test whether the nuclear activity might be involved in RNA processing, we prepared two additional synthetic RNAs with the nuclear extract. These RNAs had the identical sequence around the stem-loop but the stem-loop was internal in the transcript. These RNAs differed from each other in the sequences further downstream of the stem-loop. One (+AG) has the purine rich sequence normally found downstream of the stem-loop in the wildtype pre-mRNA and hence is a substrate for 3' processing. The other (-AG) has polylinker sequences replacing the purine rich sequence and is not a processing substrate. Mobility shift assays were done with these RNAs as well as with the 30 nucleotide RNA. Since the longer transcripts showed much more non-specific binding, it was necessary to include heparin in the binding assay and to treat the complexes with RNase prior to analysis to observe specific complexes.

Figure 5A shows that there is an activity in the nucleus which binds the internal stem-loop, resulting in a complex which after RNase treatment has identical mobility with the complex formed with the 30 nucleotide substrate (Fig. 5A, cf. lane 5 with lanes 2 and 8). This complex is specific for the stem-loop since incubation with excess unlabeled 30-mer containing only the stem-

Figure 5. Binding of longer RNA substrates with internal stem-loop sequences. A. 160 nt radiolabeled RNA substrates containing the wild-type 3' end of the H2a-614 gene (+AG, lanes 1–3) or an identical RNA except for the removal of the purine-rich U7 snRNP binding site removed (-AG, lanes 7–9) were incubated with 68 μ g of protein from a nuclear extract as described in Fig. 2, except that 50 μ g/ml heparin was included in the incubation. The complexes were treated with RNase A (100 μ g/ml for 5 min) on ice and then analyzed by electrophoresis. The SL_{wt} probe was incubated in the absence (lane 4) or presence (lanes 5 and 6) and the complexes formed on the 30 nt stem-loop was analyzed using the same procedure. A 100-fold molar excess of unlabeled 30nt stem-loop was included as a specific competitor in lanes 3, 6 and 9. Lanes 4–6 are from the same gel exposed for one-fourth as long as the other lanes. N indicates the position of the specific complex and RNA the position of the input probe. B. The same substrates were incubated with 68 μ g of protein from a nuclear extract (lanes 1–3, 6–8) or with 30 μ g of protein extracted from polyribosomes (lanes 4 and 9), and assayed as described in A. Lane 5 shows incubation of the -AG substrate in buffer. In lanes 2 and 7, a 10-fold excess of competitor stem-loop sequence was included and in lanes 3 and 8, a 100-fold excess of competitor stem-loop sequence was included. The position of the complex formed with the nuclear extract is indicated (N). Most of the sample in lane 2 was lost during loading of the gel.

loop sequence the binding with the large substrate (Fig. 5A, lanes 3 and 9). The same complex was formed whether or not the U7 snRNP binding site was present (Fig. 5A, lane 8) and the mobility of the complex was similar to that formed with the 30-mer (Fig. 4, lane 5). There was 50% more probe RNA used in lanes 1–3 compared with lanes 7–9. The proportion of RNA found in the complex was similar with each of the longer probes, suggesting that the affinity of the proteins for both substrates is the same. A higher proportion of the 30-mer is found in a complex at the same protein concentration, about 3-fold higher after correcting for the number of C's in the protected complex, suggesting that the factor may have a higher affinity for RNAs in which the stem-loop is located at the 3' terminus. This conclusion is tentative due to the slightly different conditions necessary for the assays with the longer complexes.

A similar experiment was done with the polyribosomal extract using the same substrates (Fig. 5B). Again incubation of either of the longer substrates with the nuclear extract resulted in detectable complex formation (Fig. 5B, lanes 1 and 6), although the complex formation was not dependent on the presence of the U7 snRNP binding site. When the same substrates were incubated with the polyribosomal extract there was not a defined complex formed (Fig. 5B, lanes 4 and 9), although there was a small amount of a broad band of slightly lower mobility than the probe, present using both probes. About 5 times as much binding activity was present in the polysomal extracts compared to the nuclear extracts when analyzed using the normal stem-loop probe (not shown), suggesting that the polysomal extract had a very low affinity for the longer transcript. Thus the polysomal activity also binds the stem-loop sequence at the 3' end of the RNA better than the internal stem-loop sequence.

These data show that there is a specific protein(s) which can interact with the stem-loop at the 3' end of histone mRNA found in both the polyribosomes and the nuclear fraction, and that the protein binds better to a stem-loop sequence located at the 3' end of the mRNA.

DISCUSSION

Most mRNAs end in a poly(A) sequence whose function is not completely understood, although it clearly plays a role in determining the half-life of the mRNA and may also play a role in the translation of the mRNA (27). The poly(A) sequence is bound to a specific protein, the poly(A) binding protein, which is a component of the mRNP (28). The 3' end of histone mRNA is required for the regulation of histone mRNA half-life (6,7) and hence takes on one of the roles of poly(A) binding protein.

It seems likely that a protein bound to the 3' end of histone mRNA is a component of the histone mRNP. We have found that there is an activity in polyribosomes and in the nucleus which specifically interacts with the 3' end of histone mRNA. There is no detectable activity in the soluble fraction of the cytoplasm (not shown). This is in contrast to the protein which both regulates the stability of the transferrin receptor mRNA and the translation of the ferritin mRNA which is found soluble in the cell (10). Similarly a protein which interacts with the AU-rich sequences characteristic of unstable mRNAs, is also found soluble in the cytoplasm (29,30).

Possible functions of the stem-loop binding protein(s)

These results suggest that specific binding activities which recognize the 3' end of histone mRNA are present in both the

polyribosomes and the nucleus. Although the complexes differ in electrophoretic mobility, the proteins detected by UV-crosslinking are similar in size, raising the possibility that the same polypeptides are in contact with the histone mRNA in both the nucleus and the polyribosomes. In support of this possibility is the finding that the specificity of the binding activities in the nucleus and the cytoplasm are similar with different analogues of the stem-loop sequence. It is certainly possible that there are different proteins in the cytoplasm and nucleus which bind the stem-loop, as there are different nuclear and cytoplasmic poly(A) binding proteins (31,32).

It is likely that the stem-loop binding protein found in the polyribosomes is part of the histone mRNA and plays a role analogous to that of the poly(A) binding protein, which is been implicated in both mRNA stability (33,34) and translation (27,35,36). The 3' end of the histone mRNA is required for regulation of mRNA stability (6,7) and the histone mRNA is degraded 3' to 5' (14,37). Thus the polysomal protein could play a role in regulating histone mRNA stability, perhaps by protecting the histone mRNA from degradation. Accelerated degradation which occurs when DNA synthesis is inhibited (38) or at the end of S-phase (39), might then require prior removal of the protein from the histone mRNA.

The possible function of the nuclear protein is less clear. It could be part of the stem-loop binding factor (26) which is one of the components required for formation of the 3' end of histone mRNA. The protein binds weakly to the stem-loop even when the stem-loop is internal in the transcript, although there is no dependence on the presence of a U7 snRNP binding site for the binding. Alternatively, since this activity binds strongly to RNAs with the stem-loop at the 3' end, the structure found on mature histone mRNA, the nuclear protein might associate with the 3' end of the histone mRNA only after processing has occurred. The protein might then be involved in recognizing the newly processed histone mRNA and in aid in transport to the cytoplasm. If indeed the same proteins are bound to the stem-loop in the nucleus and the polyribosomes, then a component of the nuclear complex would become part of the histone mRNP. The protein could shuttle to the nucleus upon destruction of a histone mRNA and participate in the transport of another histone mRNA. Elucidation of the exact function of these RNA-protein complexes awaits purification of the protein(s).

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