Changes in the stem-loop at the 3' terminus of histone mRNA affects its nucleocytoplasmic transport and cytoplasmic regulation

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Received July 19, 1994; Revised and Accepted September 24, 1994

ABSTRACT

The stem-loop structure at the 3' end of replication-dependent histone mRNA is required for efficient pre-mRNA processing, localization of histone mRNA to the polyribosomes, and regulation of histone mRNA degradation. A protein, the stem-loop binding protein (SLBP), binds the 3' end of histone mRNA and is thought to mediate some or all of these processes. A mutant histone mRNA with two nucleotide changes in the loop was constructed and found to be transported inefficiently to the cytoplasm. The mutant histone mRNA, unlike the wild-type histone mRNA, was not rapidly degraded when DNA synthesis is inhibited, and was not stabilized upon inhibition of protein synthesis. The stem-loop binding protein (SLBP) has between a 20–50 fold greater affinity for the wild type histone stem-loop structure than for the mutant stem-loop structure, suggesting that the alteration in the efficiency of transport and the normal degradation pathway in histone mRNA may be due to the reduced affinity of the mutant stem-loop for the SLBP.

INTRODUCTION

During S-phase in mammalian cells DNA replication and synthesis of the core histone proteins occur coordinately. The expression of the replication-dependent histone genes is regulated at both the transcriptional and post-transcriptional level during the cell cycle (1,2). Replication-dependent histone genes do not contain introns and their transcripts are not polyadenylated, but instead end in a stem-loop structure that has been highly conserved among all metazoan histone mRNAs (3). The only processing reaction in histone mRNA biosynthesis is the endonucleolytic cleavage between the stem-loop sequence and the purine-rich element which binds U7 snRNP (4,5). The steady-state levels of replication-dependent histone mRNAs are 30–40

fold higher in S-phase than in G₁-phase, due to an increase in the transcription rate and the efficiency of 3' end formation (1). In G₂-phase the histone mRNA is rapidly degraded and this rapid degradation requires the 3' stem-loop sequence (6) and continued protein synthesis (7,8). Histone mRNA is degraded 3' to 5', probably by a ribosome bound nuclease (9,10).

The 3' end of histone mRNA has been implicated in the transport of histone RNA to the cytoplasm and localization to polyribosomes (11,12). Little is known about the specific RNA sequences or protein factors required for nucleoplasmic transport of mRNAs. The snRNAs synthesized by RNA polymerase II require a 5' monomethylated guanosine cap [(5')m⁷Gppp(5')N] and a cap binding protein for efficient export (13,14). Additional sequences (structures) in the snRNA which promote transport out of the nucleus are also required but have not been completely defined yet (15). Eckner and Birnstiel have reported that formation of histone 3' ends by the normal processing pathway is necessary for transport of histone mRNA (12). We have previously reported that the 3' end of histone mRNA is necessary for efficiently localizing the histone mRNA to polyribosomes (11). This could reflect either a role for the 3' end in transport or translation of histone mRNA.

Recently we have described a 45 kD stem-loop binding protein (SLBP), which specifically binds to the 3' end of histone mRNA, that is present in both the nucleus and cytoplasm. The cytoplasmic SLBP is found on polyribosomes, presumably as a component of the histone mRNP (16). In the experiments reported here, we have expressed a histone mRNA with a mutant stem-loop which has a greatly reduced affinity for the SLBP. We find that this mRNA is defective in several aspects of its nuclear and cytoplasmic metabolism: it is exported inefficiently from the nucleus and is not rapidly degraded when DNA synthesis is inhibited. The simplest explanation for these results is that proper stem-loop RNA–SLBP interactions are required for efficient nuclear-cytoplasmic transport and proper regulation of histone mRNA degradation.

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MATERIALS AND METHODS

Histone gene constructs

The cloned mouse histone H2a-614 gene in pUC18 has been described previously (17). It was used to construct the H2aSL(A^{1,3})SL_{wt} gene that has two processing signals (18). The first processing signal is a mutant 3' stem-loop, [SL(A^{1,3})], in which adenosines are substituted for the first and third uridines in the loop, and the second wild-type histone processing signal is 60 nucleotides downstream. Both processing signals contain the U7 binding site present in the H2a-614 gene. The wt and mutant A^{1,3} stem-loop structures and the structure of the H2aSL(A^{1,3})SL_{wt} gene are shown in Figure 1.

Cell culture and transfections

The mouse histone genes were introduced together with the pSV₂-neo gene into Chinese Hamster Ovary (CHO) cells using the polybrene method of Chaney *et al.* (19). Each transfection yielded at least 20 to 50 independent G-418 resistant colonies which were expanded into mass cultures. Exponentially growing cultures were fed with fresh media 8–12 h before starting an experiment. Total RNA was extracted as previously described (20). For studies of histone mRNA stability, cells were treated with either 5 mM hydroxyurea or 100 mM cycloheximide for 60 min prior to isolation of RNA. Cells were fractionated into nuclear, polyribosomal and cytosolic fractions and RNA prepared from each fraction as previously described (11).

Analysis of RNA

To measure the level of histone mRNA, the H2aSL(A^{1,3})SL_{wt} gene was labeled with [α -³²P]-dCTP at the 3' end of the *Ava*I site at codon 20, and the amounts of both the endogenous hamster histone H2a mRNA and the transfected mRNA were measured by S1 nuclease protection (18). The S1-resistant fragments were resolved by electrophoresis on 6% polyacrylamide gels containing 7M urea and following autoradiography, the relative amounts of each protected fragment were determined by densitometric scanning or using a PhosphorImager (Molecular Dynamics).

For analysis of U1 snRNA, a riboprobe was synthesized from a cloned *Xenopus* U1 snRNA gene (21) using T7 RNA polymerase. The riboprobe extended from -363 to +120. 500 ng of RNA was analyzed as described previously for the analysis of mouse U1 snRNA (22).

Injection of *Xenopus* oocytes

Supercoiled DNA (15 nl, 30 μ g/ml) was injected into stage VI *Xenopus* oocytes (23) and the oocytes were incubated at 18°C for 18 h. RNA was prepared from whole oocytes or from cytoplasmic and nuclear fractions. For fractionation, the oocytes were fixed in acetone and dissected manually in acetone. Residual acetone was removed by heating the oocytes at 65°C degrees for approximately 5 min. RNA was extracted as previously described (24). The RNAs were analyzed for histone mRNA (S1 nuclease mapping), for U1 RNAs [riboprobe assay (22)] and for ribosomal RNA (agarose gel electrophoresis).

RNA–protein binding and competition assays

A 30 nt RNA probe containing the wild-type stem-loop sequence was synthesized using oligonucleotide templates, T7 RNA polymerase and [α -³²P]-UTP (25). For competition experiments, an unlabeled 30 nucleotide wt and mutant stem-loop was synthesized using T7 RNA polymerase and purified by

preparative gel electrophoresis, using the appropriate oligonucleotide template to synthesize the SL(A^{1,3}) RNA. 3×10^{-15} moles of labeled wild-type stem-loop RNA probe was used in each 30 μ l binding reaction. The wild-type probe together with the wild type or mutant unlabeled competitors were mixed together before addition of the nuclear extract. 10 μ g of nuclear proteins prepared from exponentially growing mouse myeloma cells were incubated with the synthetic RNAs and 60 μ g yeast tRNA for 20 min on ice (16). The complexes were resolved on 6% non-denaturing polyacrylamide gels containing 3.3 mM NaOAc, 1 mM EDTA and 6.7 mM Tris–HCl, pH 8.0. The relative intensities of the bands were quantified using a PhosphorImager (Molecular Dynamics).

RESULTS

The six base-pair stem and four base loop structure at the 3' end of histone mRNA has been highly conserved in metazoans (3) and is shown in Figure 1A. The loop has a conserved UYUN sequence while the stem has also been conserved with an UA base-pair at the top of the stem, two GC base pairs at the base of the stem and 3 pyrimidine–purine base pairs between these

Figure 1. The structure of the histone mRNA 3' end and the H2aSL(A^{1,3})SL_{wt} gene. **A.** Shown here is the consensus structure of the 3' end of histone transcripts, including the stem-loop sequence and 5 nts flanking the stem-loop on either side derived from 100 sequences of vertebrate histone mRNAs and the consensus U7 snRNA binding site (underlined), taken from Bond *et al.* (43). The nts which have been 100% conserved among the vertebrate histone mRNAs are boxed. **B.** The structure of the H2aSL(A^{1,3})SL_{wt} gene (18) is shown. The small blocks are the 5' and 3' untranslated regions. The large block is the complete H2a-614 coding region. Beneath the gene is the sequence of the 3' end of the H2a-614 wild-type and mutant H2aSL(A^{1,3}) mRNAs.

conserved elements. All species have a uridine in the first and third position of the loop except *C.elegans* which has a cytosine at position 1 in the loop (26). The sequences flanking the stem-loop on either side have also been conserved. The conserved features are shown in Figure 1A and the conserved nucleotides are boxed. Most mutations in the stem-loop structure, including changing the 1st and 3rd nts in the loop from U to A [SL(A^{1,3}), Fig. 1B] abolish expression of histone mRNA, due to a failure to efficiently process the pre-mRNA (18). We have recently shown that the expression of this mutant histone mRNA can be 'rescued' by placing a wild-type processing site downstream of the mutant processing site (18). Thus the H2aSL(A^{1,3})SL_{wt} gene (Fig. 1B) produces two mRNAs, one ending at the mutant (A^{1,3}) stem-loop and a 97 nt longer transcript ending at the wild-type stem-loop. In CHO cells, 90–95% of the transcripts from this gene in CHO cells end at the wild-type stem-loop and the ratio of wild-type to mutant transcripts is 17:1 (see Fig. 3). Thus, by expressing two different transcripts from the same gene, we can compare the metabolism of histone transcripts differing at their 3' ends.

The H2aSL(A^{1,3}) mRNA is not transported efficiently in rodent cells

CHO cells transfected with the H2aSL(A^{1,3})SL_{wt} gene were fractionated into nuclear (N), polyribosomal (P) and cytosolic (S) compartments and RNA prepared from each sample. More than 90% of the endogenous hamster histone H2a mRNA and the mRNA from the transfected gene ending at the wild-type stem-loop were present in the polyribosomal fraction (Fig. 2A, lanes 1–3). Since there is only a small amount of H2aSL(A^{1,3}) produced, we analyzed larger amounts of nuclear RNA in parallel with the same amounts of cytoplasmic and cytosolic RNA. If the H2aSL(A^{1,3}) mRNA is colocalized with the wild-type histone mRNA, then the relative amounts of the two RNAs will be identical in each cell fraction. The ratio of the wild-type transcript to the mutant H2aSL(A^{1,3}) transcript was 3:1 in the nuclear fraction and much higher, 20:1, in the cytoplasmic fraction (Fig. 2A, lanes 4 and 5). We estimate that 25–30% of the H2aSL(A^{1,3}) mRNA is in the nucleus in the steady-state RNA. The presence of a larger proportion of the H2aSL(A^{1,3}) transcripts in the nucleus is consistent with a reduced export of

Figure 2. Localization of the transcripts from the H2aSL(A^{1,3})SL_{wt} gene. **A.** CHO cells stably transfected with the H2aSL(A^{1,3})SL_{wt} gene were fractionated into nuclear (N, lanes 1 and 4), polyribosomal (P, lanes 2 and 5) and cytosolic (S, lanes 3 and 6) fractions and the transcripts measured by S1 nuclease mapping using the H2aSL(A^{1,3})SL_{wt} gene labeled at the 3' end of the *Ava*I site at codon 20 as a probe. In lanes 1–3 RNAs from equal numbers of cells were analyzed and the gel was exposed to show the localization of the endogenous hamster histone mRNAs and the transcripts ending at the wild-type H2a-614 stem-loop. In lane 4, nuclear RNA from twice as many cells was analyzed as in lanes 5 and 6 to allow the transcripts of the mouse genes to be readily visualized. This gel was exposed for 10 times longer than the gel in lanes 1–3. The protected fragments are H2a_H — protection of the endogenous hamster H2a histone mRNAs; H2a_{M-SL_{wt}} — protection of the transcripts from the transfected gene ending at the wild-type stem-loop; H2a_{M-SL(A^{1,3})} — protection of the transcripts from the transfected gene ending at the SL(A^{1,3}) stem-loop. **B.** Supercoiled DNA (15 nl, 30 µg/ml) was injected into stage VI *Xenopus laevis* oocytes (23) and the oocytes were incubated at 18°C for 8 (lanes 6 and 7) or 18 h. RNA was prepared from whole oocytes (W, lane 3) or from oocytes fractionated into nuclear (N, lanes 4 and 6) and cytoplasmic (C, lanes 5 and 7) fractions. RNA from equal numbers of oocytes in each lane was analyzed as described in panel A. Lane 2 is analysis of 10 µg of yeast tRNA. Lane 1 is pUC18 digested with *Hpa*II. The protected fragments are labeled as in panel A. The size of the marker in nts is given. The S1 nuclease assay is diagrammed below the figure.

Table 1. Delayed transport of the SL(A^{1.3}) histone mRNA

mRNA	8 h		18 h	
	Nuclear	Cytoplasm	Nuclear	Cytoplasm
SL(A ^{1.3})SL _{wt}	33	67	15	85
SL(A ^{1.3})	60	40	25	75

The H2a SL(A^{1.3})SL_{wt} gene was injected into *Xenopus* oocytes and RNA prepared from oocytes fractionated into nuclear and cytoplasmic fractions 8 or 18 h after injection of the DNA. The proportion of the two mRNAs in each fraction was measured by S1 nuclease mapping as in Figure 2B. The results are expressed as per cent of each RNA in the particular subcellular fraction at the indicated time.

the mutant transcripts from the nucleus to the cytoplasm. These fractionation studies were done in triplicate and the results were essentially identical in each fractionation.

The mutant H2aSL(A^{1.3}) mRNA is not transported efficiently in *Xenopus* oocytes

The fate of the transcripts of the H2aSL(A^{1.3})SL_{wt} gene was also followed in *Xenopus* oocytes, a system in which the cells can be readily fractionated into nuclear and cytoplasmic fractions. When the gene was injected into frog oocytes, transcripts which ended at the mutant SL(A^{1.3}) stem-loop were formed (Fig. 2B, lane 3). The relative proportions of the two transcripts varied depending on the batch of oocytes, but in general a larger fraction of the transcripts ended at the SL(A^{1.3}) stem-loop in *Xenopus* oocytes than in CHO cells (see Fig. 2B, lanes 3–5 and lanes 6 and 7 for example). The relative amounts of the two transcripts was analyzed at 8 and 18 h after injection. After 18 h the majority of the transcripts remaining in the nucleus end at the mutant stem-loop (Fig. 2B, lane 4), while the majority of the cytoplasmic transcripts end at the wild-type stem-loop (Fig. 2B, lane 5). In the total RNA sample there was an intermediate ratio of the two transcripts as expected (Fig. 2B, lane 3). In a second experiment with a different batch of oocytes, similar results were obtained with oocytes incubated for 8 h; a higher proportion of the mutant RNA was in the nuclear fraction, while the majority of the SL(A^{1.3})SL_{wt} RNA was in the cytoplasm (Fig. 2B, lanes 6 and 7). In control experiments the wild-type histone H3-614 gene and the H2aSL(A^{1.3})SL_{wt} gene were coinjected and the H3-614 mRNA was transported identically with the SL(A^{1.3})SL_{wt} mRNA, showing that the longer mRNA ending with a wild-type histone 3' end was transported normally and was not affected by the presence of the mutant stem-loop sequence in the transcript (data not shown). These results are summarized in Table I. The SL(A^{1.3}) histone mRNA was transported to the cytoplasm more slowly than the wild-type histone mRNA, although after 18 h most of the mutant mRNA was transported.

The H2aSL(A^{1.3}) mRNA is less sensitive to inhibition of DNA synthesis

Since 75% of the steady-state SL(A^{1.3}) RNA was found on polyribosomes in CHO cells, we tested the stability of the SL(A^{1.3}) RNA in response to two agents which affect histone mRNA degradation, hydroxyurea (HU), an inhibitor of DNA replication and cycloheximide, an inhibitor of protein synthesis. Replication-dependent histone mRNAs normally have a half-life of 45 to 60 min during S phase (27,28), and when S-phase cells are treated with inhibitors of DNA synthesis, the half-life of histone mRNA is reduced to 8–10 min (27,29). Furthermore, treatment of cells with HU reduces histone mRNA levels about 20-fold in 60 min, due both to a decrease in the rate of histone

gene transcription and an increase in the rate of histone mRNA degradation (27,30).

Cells transfected with the H2aSL(A^{1.3})SL_{wt} gene were treated with hydroxyurea for 60 min. The amounts of the endogenous hamster H2aH mRNA and the transcripts ending at the wild-type stem-loop were reduced 18-fold after treatment with HU for 60 min (Fig. 3A, cf. lanes 3 with 4 and 5), while the amount of the mutant SL(A^{1.3}) mRNA was only reduced 4 to 5-fold (Fig. 3A, cf. lanes 3 with 4 and 5). As a better indication of the difference in response to HU, we measured the relative levels of the H2aSL(A^{1.3})SL_{wt} and the H2aSL(A^{1.3}) mRNA in the same lane. This ratio changed from 17:1 in the control cells to 5:1 in the HU treated cells. This change in ratio is entirely consistent with the interpretation that the SL(A^{1.3}) RNA is not rapidly degraded after treatment with HU. The 5-fold decrease in the amount of SL(A^{1.3}) RNA after HU treatment was likely due to the decrease in transcription as a result of the HU treatment (27). It is possible that the mutant mRNA was also destabilized after treatment with hydroxyurea, although to a lesser extent than the wild-type histone mRNA.

The H2aSL(A^{1.3}) mRNA is not stabilized by treatment with cycloheximide

Inhibition of protein synthesis also alters histone mRNA half-life by stabilizing histone mRNA (1), resulting in an increase in histone mRNA levels. Treatment of cells with cycloheximide for 60 min resulted in a 2 to 3-fold increase in the level of the endogenous hamster (H2aH) and H2aSL(A^{1.3})SL_{wt} mRNAs, while the level of the H2aSL(A^{1.3}) mRNA did not change (Fig. 3B, cf. lanes 1 and 2). In order to see the 2 to 3-fold increase in endogenous hamster H2a mRNA levels, a lighter exposure of the same sample is shown (Fig. 3B, lanes 5 and 6). Identical results were obtained with pactamycin, which inhibits protein synthesis by blocking translation initiation (not shown). These results provide further evidence that the regulation of the H2aSL(A^{1.3}) mRNA is abnormal.

The stem-loop binding protein (SLBP) has a reduced affinity for SL(A^{1.3}) stem-loop

We have shown previously that specific RNA–protein complexes are formed on the wild-type stem-loop sequence in both the nucleus and polyribosomes from mouse myeloma cells (16). These complexes share a common 45 kD protein (the SLBP). Similar complexes are formed in extracts from frog oocytes (our unpublished results). As measured by the mobility shift assay, no detectable binding of the SL(A^{1.3}) RNA to either the nuclear or the polyribosomal SLBP occurred (16). However, it was possible to compare the relative affinities of the SLBP for the SL(A^{1.3}) and wild-type sequence using competition experiments. Binding of the radiolabeled 30 nt wt stem-loop RNA probe by

Figure 3. Effect of metabolic inhibitors on levels of transcripts of the H2aSL(A^{1.3})SL_{wt} gene. **A.** RNA was prepared from CHO cells transfected with the H2aSL(A^{1.3})SL_{wt} gene (lanes 3 and 7) and from cells treated with 5 mM hydroxyurea for 60 min (lanes 4, 5, 8 and 9). 30 µg of RNA was analyzed by S1 nuclease mapping as in Figure 2. Three and 30 µg of RNA from untransfected CHO cells (lanes 1 and 6) and 3 µg of RNA from CHO cells transfected with the H2a-614 gene (lane 2) were also analyzed. Lane M is pUC18 digested with *Hpa*II. The protected fragments are labeled as in Figure 2A. Lanes 7-9 are a shorter exposure of lanes 3-5 showing the effect of hydroxyurea on the endogenous hamster histone H2a mRNA. The amounts of the different mRNAs were quantified by densitometry using different exposures of the same gel. **B.** RNA was prepared from CHO cells transfected with the H2aSL(A^{1.3})SL_{wt} gene (lanes 1 and 5) and from cells treated with 0.1 mM cycloheximide for 60 min (lanes 2 and 6). Thirty µg of RNA from transfected (lanes 1, 2, 5 and 6) and untransfected (lanes 3 and 7) cells was analyzed by S1 nuclease mapping as in Figure 2. The protected fragments are labeled as in Figure 2A. Lanes 4 and 8 are analysis of 10 µg of yeast tRNA. Lane M is pUC18 digested with *Hpa*II. Lanes 5-8 are a lighter exposure (1/10 the time) of the same gel shown in lanes 1-4. The fragment labeled P corresponds to the probe.

the SLBP was competed with increasing amounts of either unlabeled wt or SL(A^{1.3}) RNA. The unlabeled wild-type stem-loop RNA competed efficiently (Fig. 4, lanes 2-6), while 20-50 times as much unlabeled SL(A^{1.3}) RNA as wild-type RNA was required for the same degree of inhibition (Fig. 4, lanes 8-12). Thus we estimate that the SL(A^{1.3}) RNA binds the SLBP with 20-50-fold lower affinity than the wild-type stem-loop RNA.

DISCUSSION

These experiments show that a histone mRNA which ends in a mutant stem-loop structure is metabolized abnormally. In addition to inefficient processing of the mutant pre-mRNA (18), it is transported inefficiently from the nucleus and its half-life in the cytoplasm is not regulated properly. The fact that the SL(A^{1.3})

Figure 4. Relative affinity of the wild type and mutant stem-loops for the SLBP. A radiolabeled 30 nt RNA probe containing the wild-type stem-loop sequence was incubated with a nuclear extract in the presence of increasing amounts of competitor RNA containing the wild type stem-loop sequence (lanes 2-6) or the SL(A^{1.3}) stem-loop sequence (lanes 8-12). The complexes were resolved by gel electrophoresis under non-denaturing conditions. The specific complex is labeled N and there is a small amount of non-specific complex labeled ns. The numbers above the lanes refer to the fold excess of competitor present. The sequence of the two RNA probes is shown beneath the figure.

RNA sequence has a reduced affinity for the SLBP and the H2aSL(A^{1.3}) mRNA is not as efficiently transported nor degraded normally is consistent with the SLBP having a critical role in both these processes.

The final step in mRNA biogenesis is transport of the mRNA from the nucleus to the cytoplasm. Most pre-mRNAs undergo a series of splicing reactions and only the completely spliced RNA is exported to the cytoplasm. It is likely that the cell recognizes the presence of introns in the pre-mRNA and retains the RNA in the nucleus until splicing is completed (31). Specific *trans*-acting factors (e.g., the rev protein) allow the transport of incompletely spliced RNAs in some retroviruses (32-34) and the rev protein may accompany the mRNA to the polyribosomes (35). Histone pre-mRNAs lack introns and the only processing reaction is the endonucleolytic cleavage to form the histone 3' end. Since less than 5% of the histone RNA is found in the nuclear fraction and the normal half-life of the cytoplasmic mRNA is about 45 min, the transit time of histone mRNA after 3' processing in the nucleus must be less than five minutes.

One of the factors involved in 3' processing of histone mRNA interacts with the stem-loop (5,36,37). There is also a factor present in the polyribosomes bound to the stem-loop at the 3' end of the histone mRNA. The nuclear and polyribosomal factors

detected by the mobility shift and UV crosslinking are similar if not identical. Mutations in the stem-loop which alter the binding of the SLBP to the stem-loop effect the processing of the histone mRNA *in vivo* in parallel with the effects on SLBP binding (18). It is possible that the SLBP binds to the pre-mRNA before processing and accompanies the mature mRNA to the cytoplasm.

Eckner and Birnstiel have reported a requirement for the normal 3' processing reaction for transport of histone mRNA to the cytoplasm (12). Transcripts with histone mRNA 3' ends formed by a ribozyme cleavage reaction were retained in the nucleus. This effect was only observed for the CAT mRNA; transcripts formed by a ribozyme cleavage which encoded histone proteins were apparently exported normally (12). These results are not inconsistent with our results. The normal histone 3' end is important for export of mRNA from the nucleus and localization on polyribosomes, probably as a stem-loop-SLBP complex (11). If the SLBP normally associates with the histone pre-mRNA as part of the processing reaction, then the failure to export some transcripts formed by ribozyme cleavage can be understood. The reduced affinity of the SLBP for the mutant RNA may result in the inefficient transport of the mRNA, possibly due to dissociation of the SLBP prior to transport.

We conclude that the SL(A¹⁻³) mRNA is transported inefficiently in CHO cells based on the large fraction of mutant mRNA present in the nucleus in steady-state RNA. Because only steady-state RNA measurements can be made in CHO cells, it is impossible to determine whether the SL(A¹⁻³) mRNA has a longer transit time in the nucleus or whether some of the RNAs are never transported.

The altered regulation of half-life of the SL(A¹⁻³) mRNA is revealed by two types of experiments. The SL(A¹⁻³) mRNA is not as rapidly degraded after hydroxyurea treatment as the wild-type histone mRNA and the amount of SL(A¹⁻³) mRNA is not increased after cycloheximide treatment which stabilizes wild-type histone mRNA. The observed decrease in SL(A¹⁻³) mRNA after hydroxyurea treatment can be accounted for solely by the decrease in transcription of the gene. The mechanism of stabilization of histone mRNA (and many other short-lived RNAs) by inhibition of protein synthesis is not known. However, not all mRNAs are stabilized by inhibiting translation, and the sequences involved in stabilization by cycloheximide and those that determine the half-life of some mRNAs are separable (38-40). Note that our results cannot be accounted for by the slightly shorter half-life (25 min vs. 50 min, as judged by actinomycin D treatment) of the SL(A¹⁻³) mRNA compared with the wild-type mRNA (18). The levels of an mRNA with a short half-life might not be affected as much by hydroxyurea treatment since the change in half-life might not be as dramatic. However, the levels of a less stable mRNA would be increased more than the levels of the wild-type mRNA after stabilization by inhibition of protein synthesis. Since much (75%) of the SL(A¹⁻³) mRNA is ultimately present on polyribosomes where histone mRNA degradation occurs (41,42), the altered degradation must be a consequence of the change in the sequence of the 3' end of the mRNA.

It is possible that all three defects (inefficient processing, reduced transport and altered degradation) of the mutant mRNA may be mediated by a common mechanism, and may be due to the reduced affinity of the mutant stem-loop for the SLBP. The SLBP has been detected in the nuclei and polyribosomes of mammalian cells (16,18) and a similar activity has been detected in extracts of frog oocytes (unpublished results). The mutant 3'

end is defective in binding the SLBP, with a 20-50-fold lower affinity. The binding of the SLBP to the wild type histone 3' end is very stable, with a half-life of over 2 h *in vitro* (our unpublished results). One consequence of the reduced affinity for the SLBP would be an increased off-rate, resulting in the loss of the SLBP from the mRNA either before or after transport from the nucleus. We suggest that the SLBP may play an important role not only in histone mRNA processing, but also in transport and cytoplasmic metabolism of the histone mRNA.

ACKNOWLEDGEMENTS

This work was supported by NIH grants GM27789 to W.F.M and HL42250 to B.K.K. T.C.I. was supported by NIH Training Grant GM07092 to the Curriculum in Genetics and Molecular Biology.

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