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Human La Protein: a Stabilizer of Histone mRNA

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Histone mRNA is destabilized at the end of S phase and in cell-free mRNA decay reaction mixtures supplemented with histone proteins, indicating that histones might autoregulate the histone mRNA half-life. Histone mRNA destabilization in vitro requires three components: polysomes, histones, and postpolysomal supernatant (S130). Polysomes are the source of the mRNA and mRNA-degrading enzymes. To investigate the role of the S130 in autoregulation, crude S130 was fractionated by histone-agarose affinity chromatography. Two separate activities affecting the histone mRNA half-life were detected. The histone-agarose-bound fraction contained a histone mRNA destabilizer that was activated by histone proteins; the unbound fraction contained a histone mRNA stabilizer. Further chromatographic fractionation of unbound material revealed only a single protein stabilizer, which was purified to homogeneity, partially sequenced, and found to be La, a well-characterized RNA-binding protein. When purified La was added to reaction mixtures containing polysomes, a histone mRNA decay intermediate was stabilized. This intermediate corresponded to histone mRNA lacking 12 nucleotides from its 3′ end and containing an intact coding region. Anti-La antibody blocked the stabilization effect. La had little or no effect on several other cell cycle-regulated mRNAs. We suggest that La prolongs the histone mRNA half-life during S phase and thereby increases histone protein production.

Expression of the replication-dependent histone genes is regulated at the transcriptional and posttranscriptional levels in such a way that histone protein synthesis is restricted to S phase (reviewed in references 22, 34, 35, 44, 60, and 61). Histones are not synthesized during G_1 but are made at high rates during S, and histone mRNA levels reflect these changes in histone synthesis. At the end of S, the mRNA rapidly disappears from the cytoplasm as a result of three processes: reduced histone gene transcription, reduced histone pre-mRNA processing, and accelerated histone mRNA decay. This multilevel down-regulation scheme presumably ensures that free histones will not accumulate and cause toxicity during mitosis and G_1.

We have been investigating histone mRNA destabilization by using a cell-free mRNA decay system that includes polysomes and postpolysomal supernatant (S130). Polysomes with or without S130 are incubated for various times, and the half-lives of polysome-associated mRNAs are determined (56). mRNAs such as histone are unstable in cells and in this system. mRNAs that are stable in cells are stable in vitro. Our histone studies have focused on an autoregulation model to account for the post-S destabilization of histone mRNA. The model suggests that the destabilization process is triggered by the transient accumulation of histone proteins in the cytoplasm (1, 6, 39, 52, 59, 67). The following observations and correlations support this model. (i) Histone proteins accumulate in the cytoplasm after DNA synthesis stops (3, 43, 62, 63). Perhaps newly translated histones migrate rapidly to the nucleus during S but stay in the cytoplasm after DNA synthesis stops. (ii) Histone mRNA is destabilized three- to sixfold soon after DNA synthesis inhibitors are added to cells (1, 2, 13, 16, 21, 59, 64). This phenomenon presumably mimics what happens at the end of S phase. (iii) The mRNA is not destabilized when DNA and protein synthesis are inhibited simultaneously. This result is predicted by the autoregulation model. Although DNA replication ceases, the mRNA is not destabilized because the histones required to induce destabilization do not accumulate when translation is blocked. (iv) In our cell-free mRNA decay system, polysome-associated histone mRNA is inherently unstable but is further destabilized approximately fourfold by addition of histone proteins (39, 51, 52, 56–58).

The specificity and mechanism of histone mRNA destabilization have been addressed in some detail. Specificity is achieved primarily via the unique 3′-terminal structure of histone mRNA. The 3′ terminus lacks poly(A) and contains a 6-bp stem and 4-base loop (29, 31, 45) (see Fig. 1B). The stem-loop is essential for proper cell cycle regulation and is the site at which mRNA degradation begins (4, 14, 29, 31, 39, 47, 56, 57, 65, 68, 75). It is also a binding site for a unique stem-loop binding protein (40, 46, 72, 73, 76). In our cell-free mRNA decay system, three factors are required to destabilize polysome-associated histone mRNA: polysomes, histones, and S130. Histone addition does not destabilize other mRNAs, while protamines and other basic proteins do not destabilize histone mRNA (39, 52). The molecular mechanism for the destabilization is unknown. Perhaps the 3′-terminal region becomes more susceptible to a histone mRNA-degrading 3′-to-5′ exoribonuclease (7, 56).

Any model explaining autoregulation in vitro must account for the requirement for the S130. One simple model involves complex formation between histones and an S130 factor. However, analyses with S130 that was fractionated on a histone-agarose affinity column led us to conclude that the S130 actually contains two autoregulatory components: a stabilizer and a histone-activatable destabilizer. The combined actions of both components account for histone autoregulation. Here we describe some properties of the stabilizer, its purification, and its identification as La.

MATERIALS AND METHODS

Cell culture, polysome and S130 isolation, and in vitro mRNA decay assays. K562 erythroleukemia cells were maintained and polysomes and S130 were prepared as previously described (52, 56, 57). A mixture of the four bovine core histones was obtained from Sigma Chemical Co. Cell-free mRNA decay reaction

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mixtures contained polysomes from 4×10^6 cells (ca. 7 μg of RNA) in a 20-μl final volume (52, 57). S130 and the material that did or did not bind to a histone-agarose column (B for bound and U for unbound) were present at a cell equivalents ratio of 1:1 with polysomes, unless indicated otherwise. Where noted, reaction mixtures also contained 200 μg of normal human serum or human autoimmune serum (designated Gore; kindly provided by Jack Keene). Reaction mixtures containing human serum were supplemented with a twofold excess of RNasin (Promega) to block serum RNase A-like activity. All the components except polysomes were mixed on ice. The polysomes were added last, and the reaction mixtures were preincubated on ice for 30 min and then incubated at 20°C for different times, at which they were plunged into dry ice. Total RNA was prepared by adding 300 μl of urea lysis buffer (7 M urea, 2% [wt/vol] sodium dodecyl sulfate [SDS; not recrystallized], 0.35 M NaCl, 1 mM EDTA, 10 mM Tris · Cl [pH 8.0] at room temperature) to each frozen sample and extracting twice with phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol/vol) and once with chloroform-isoamyl alcohol (24:1, vol/vol). RNA was concentrated by ethanol precipitation with 25 μg of carrier *E. coli* tRNA, and the pelleted RNA was rinsed with 70% ethanol, dried, resuspended in 50 μl of NET buffer (100 mM NaCl, 1 mM EDTA, 10 mM Tris · Cl [pH 7.2]), and stored at −80°C.

Polysomal RNA was analyzed by S1 nuclease protection with a 3′-end-labeled [^{32}P]DNA probe complementary to the 3′ terminus of human histone H4 mRNA (39) (see Fig. 1). Full-length histone mRNA protects a 104-nucleotide DNA fragment corresponding to the 3′-untranslated region and part of the coding region. Digestion products were electrophoresed for 5 h at 300 V in 10% polyacrylamide gels containing 7 M urea. Gels were dried and exposed to X-ray film (Fuji). Radioactivity in protected fragments was quantitated with a PhosphorImager (Molecular Dynamics). Translatable or undegraded histone mRNA was defined as the radioactivity in three bands: full-length mRNA and the −5 and −12 decay intermediates. The amount of translatable mRNA was quantitated exactly as follows. A single box was placed around the three bands in the time zero lane. Identical boxes were then placed in a straight line across the gel, and band intensities at each time point were compared with the time zero values.

Chromatography of S130 on histone-agarose. All purification steps were performed at 4°C. A 1- by 13-cm (10-ml) histone-agarose column (Sigma) containing 8 mg of the four bovine core histones per ml was preequilibrated with 10 column volumes of buffer A (100 mM potassium acetate, 2 mM magnesium acetate, 0.1 mM spermine, 2 mM dithiothreitol [DTT], 10 mM Tris · Cl [pH 7.6]). S130 from 1.4×10^{10} K562 cells and containing 250 mg of protein was centrifuged at $20{,}000 \times g$ for 10 min. The supernatant was passed through a 0.45-μm-pore-size cellulose-acetate filter and loaded onto the column under gravity flow. The column was washed with 4 column volumes of buffer A, and the flowthrough plus this 40-ml wash were pooled (unbound fraction [U]). Five column volumes of buffer B (2 M potassium acetate, 2 mM magnesium acetate, 0.1 mM spermine, 2 mM DTT, 10 mM Tris · Cl [pH 7.6]) were added, and the eluted material was collected (bound fraction [B]). Total protein recoveries measured by the Bio-Rad assay were consistently 150 to 160%. We cannot explain this observation, but we know that it did not result from the elution of histone proteins from the agarose. Approximately 3 to 5% of the protein and all of the RNA in the S130 was recovered in fraction B.

Purification of the histone mRNA stabilizer from material that failed to bind to histone-agarose (fraction U). Fractions U were pooled from four histone-agarose chromatography runs (1,000 mg of protein from 5.6×10^{10} cells). This pooled material was applied at 4.8 ml/h to a 2.5- by 10-cm (50-ml) S-Sepharose Fast Flow column (Pharmacia) equilibrated with 10 column volumes of buffer C (2 mM magnesium acetate, 0.1 mM spermine, 2 mM DTT, 10 mM Tris · Cl [pH 7.6]) with an ISCO WIZ low-pressure chromatography system. The column was washed with buffer C until the absorbance at 280 nm (A_{280}) reached baseline. The flow rate was then increased to 12 ml/h, and bound proteins were eluted with a 500-ml linear gradient from 0 to 100% buffer D (100% buffer D is 1 M potassium acetate, 2 mM magnesium acetate, 0.1 mM spermine, 2 mM DTT, and 10 mM Tris · Cl [pH 7.6]). Fractions of 10 ml were collected, and equine cytochrome *c* (Sigma) was added as the carrier to a final concentration of 0.2 mg/ml. Each fraction was dialyzed against buffer C, and then every other fraction plus the flowthrough were assayed for stabilizer activity (see Results). To increase sensitivity, each fraction was present at a 10-fold cell equivalent excess over polysomes.

Pooled active fractions were loaded at 24 ml/h onto a 1.14- by 2-cm (2-ml) Reactive Blue 4 agarose column (Sigma) equilibrated with buffer C. After washing with buffer C until the A_{280} returned to baseline, the column was eluted with 10 ml of buffer D and then with 10 ml of buffer B. Fractions were collected into tubes containing cytochrome *c* to give a final carrier concentration of 0.2 mg/ml, dialyzed against buffer C, and assayed for stabilizer activity at a 10-fold cell equivalent excess over polysomes.

Destabilizer from the Reactive Blue 4 agarose column was loaded at 1 ml/min onto a 0.5- by 5-cm (1 ml) MonoQ HR5/5 column (Pharmacia) equilibrated with buffer C. After washing until the A_{280} returned to baseline, proteins were eluted with a 0 to 1 M potassium acetate linear gradient over 20 column volumes and then by a step elution with 5 ml of buffer B. Gradient fractions (1 ml), unbound material, and material in the step elution were collected into tubes containing cytochrome *c* to give a final carrier concentration of 0.2 mg/ml and were dialyzed against buffer C. Gradient fractions were pooled in pairs and assayed for stabilizer activity at a 30-fold cell equivalent excess over polysomes.

To determine specific activity, mRNA decay reaction mixtures contained polysomes plus bovine serum albumin (100 μg) or polysomes plus various amounts of each fraction plus albumin, so that the total amount of protein added was 100 μg. In reactions with albumin alone, 68% of the histone mRNA was degraded. One unit of stabilizer was then defined as the amount of material required such that only 30% of the mRNA was degraded.

Protein microsequencing. Purified stabilizer protein from MonoQ was electrophoresed in a 12% polyacrylamide gel containing SDS (27) and visualized by staining in 0.1% Coomassie blue R250 in 50% methanol–10% acetic acid (vol/vol) and destaining overnight in 50% methanol–10% acetic acid. The stabilizer band was excised and analyzed by in situ tryptic digestion, peptide analysis, and microsequencing under the direction of Ken Williams (W. M. Keck Foundation, Biotechnology Resource Laboratory, Yale University).

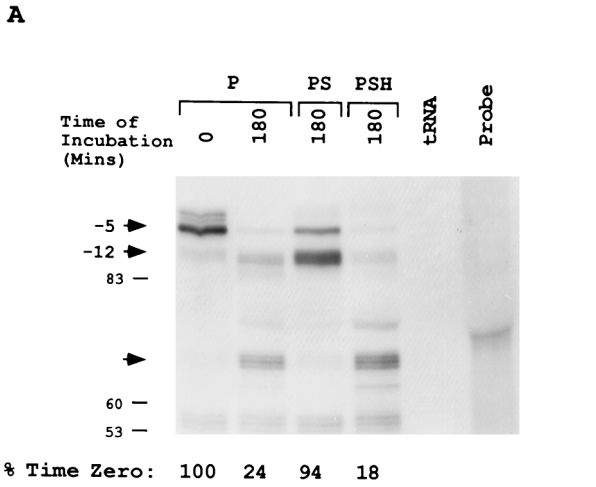
Western blot analysis. Proteins were electrophoresed in a 12% polyacrylamide gel containing SDS and transferred to nitrocellulose at 90 V for 1 h in 25 mM Tris–250 mM glycine–0.1% SDS–20% (vol/vol) methanol. The nitrocellulose was blocked overnight at 4°C in 5% dried milk in 1× Western buffer (150 mM NaCl, 0.5% [vol/vol] Nonidet P-40, 25 mM Tris · Cl [pH 8.0]). The membrane was incubated at room temperature for 1 h with anti-La monoclonal antibody SW5 (kindly provided by Walther J. van Venrooij) diluted 1:50 in 0.5% dried milk in 1× Western buffer. It was then washed four times with 1× Western buffer and incubated at room temperature for 1 h with horseradish peroxidase-conjugated anti-mouse secondary antibody (Hyclone) diluted 1:1,000 in Western buffer. The blot was then washed four more times as described above. Immunoreactive bands were detected with enhanced chemiluminescence reagents (Amersham) as specified by the manufacturer and were visualized with X-ray film.

Northern blotting. RNA was electrophoresed in a 1% agarose–2.2 M formaldehyde denaturing gel made with 10 mM sodium acetate, 1 mM EDTA, and 40 mM morpholinepropanesulfonic acid (pH 7.0). Capillary transfer to Zeta-Probe nylon membranes (Bio-Rad) and hybridization were as previously described (25, 54). Uniformly radiolabeled DNA probes were prepared by random priming from segments of cDNAs noted in the text (15). To detect 28S rRNA, blots were stripped by being washed twice for 20 min at 95°C in 50 ml of 0.1× SSC–0.1% SDS (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). They were then prehybridized for 4 h in 6× SSC–10× Denhardt's solution–0.1% SDS and hybridized overnight at 65°C in the same buffer plus 4.2 μg of a 5′-^{32}P-labeled 30-mer oligodeoxyribonucleotide (5′-ACTTTCCCTTACGGTACTTGTTGAC TATCG-3′). rRNA quantitation was then used to normalize each lane for RNA input and transfer efficiency.

RESULTS

Postpolysomal supernatant (S130) contains separable activities that stabilize or destabilize H4 histone mRNA. We reported that polysome-associated histone mRNA was at least 10-fold less stable than globin mRNA in cell-free mRNA decay reaction mixtures containing polysomes plus S130 (56). We then observed a three- to sixfold destabilization of histone mRNA when core histones were added at concentrations that could theoretically accumulate in the cell cytoplasm at the end of S phase (39, 52). Therefore, the in vitro data were consistent with the histone autoregulation model. The histones destabilized histone mRNA only in reactions with polysomes plus S130. They had no effect in reactions lacking S130. This finding prompted the work reported here to identify the required S130 factor(s).

In preliminary experiments, histone mRNA decay in reactions with polysomes alone, polysomes plus S130, or polysomes plus S130 plus histones was compared. When polysomes were preincubated for 30 min at 4°C, some full-length histone mRNA was detected, but most of the RNA was in the form of a "−5" degradation intermediate (Fig. 1A, lane P, 0; see also Fig. 1B). The −5 RNA is a previously described decay intermediate and is equivalent to histone mRNA lacking 5 nucleotides from its 3′ terminus (39). It is thought to be generated in the time zero sample by exonucleolytic nibbling of the mRNA 3′ terminus during polysome preparation and preincubation. After a 180-min incubation at 20°C, full-length histone mRNA and the −5 RNA were almost completely degraded, while two smaller RNAs appeared (Fig. 1A, lane P, 180). One was the "−12" decay intermediate, which is equivalent to histone mRNA lacking 12 nucleotides from its 3′ terminus. The other was a set of still smaller decay intermediates whose 3′ termini map 15 to 20 nucleotides 3′ of the translation termi-



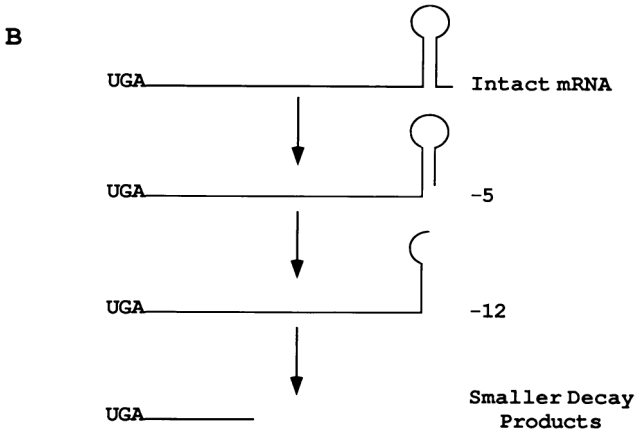


FIG. 1. An activity in postpolysomal supernatant (S130) stabilizes H4 histone mRNA. (A) Cell-free mRNA decay reactions (20 μl) contained polysomes alone (P), polysomes plus S130 (PS), or polysomes plus S130 and 4 μg of core histones (PSH). Polysomes and S130 were present at a 1:1 cell equivalency (10^6 cells/20-μl reaction mixture). Reaction mixtures were preincubated at 4°C for 30 min and then incubated at 20°C for 0 or 180 min. Total RNA was extracted and analyzed by S1 nuclease mapping with a 3′-end-labeled human H4 histone [^{32}P]DNA probe. Lane tRNA shows hybridization performed with 25 μg of *E. coli* tRNA (no cell RNA added). Lane probe contains probe DNA not treated with S1 nuclease; the undigested probe migrated above the segment of the gel shown. −5 and −12 arrows indicate DNA fragments protected by H4 histone mRNAs lacking 5 or 12 nucleotides from their 3′ ends, respectively (B). The unlabeled arrow indicates smaller decay products mapping 15 to 20 nucleotides downstream from the histone mRNA translation termination site. % Time Zero: a PhosphorImager was used to quantitate the combined radioactivity in the bands for undegraded mRNA plus the −5 and −12 decay products. The time zero value for the three combined bands was set at 100%, and the 180-min time points are shown as percent time zero. Numbers on the left are sizes (in nucleotides) of 5′-end-labeled [^{32}P]DNA markers generated by cleaving pBR322 DNA with *Hae*II. (B) Diagram of early steps in human H4 histone mRNA decay. Only the ~43-nucleotide 3′-untranslated region and translation termination codon are diagrammed. The 3′-terminal region of undegraded mRNA (top) contains a conserved 6-bp stem and 4-base loop plus 3 to 5 nucleotides on the 3′ side of the stem. The −5, −12, and smaller decay products have been detected in cells and in cell extracts (39, 52, 56, 58).

nation site (Fig. 1). The −12 RNA and smaller decay products have also been described previously (39, 56).

Adding the S130 to polysomes had two effects on histone mRNA degradation. (i) The mRNA was stabilized three- to fourfold (compare lane P, 180 with lane PS, 180). (ii) The −12 decay intermediate was the major product, and few or no smaller decay products were detected. These observations indicate that a stabilizer in the S130 slows degradation past the −12 site and thereby preserves the coding region. When polysomes, S130, and histones were incubated together, the mRNA was destabilized and degraded beyond the −12 site (compare lane PS with lanes PSH). Therefore, histone autoregulation is effected by two S130 activities: a constitutive stabilizer (Fig. 1A, lane PS) and a histone-activatable destabilizer (Fig. 1A, lane PSH). When free histones are present, the destabilizer is dominant over the stabilizer.

To determine whether either factor interacted with histone proteins, S130 was loaded onto a histone protein-agarose column at a low salt concentration. Unbound material (fraction U) was collected, and bound material (fraction B) was eluted

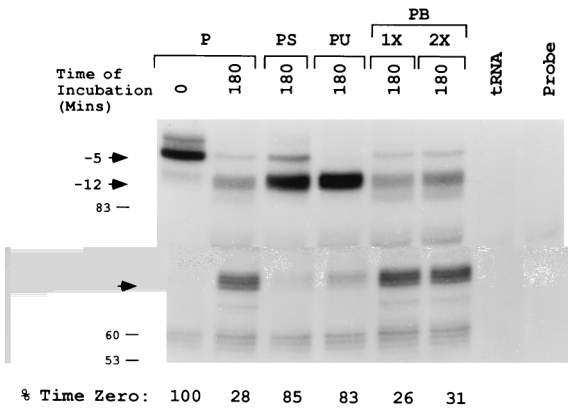


FIG. 2. Identification of a stabilizer activity in the unbound (U) fraction of a histone-agarose column. S130 was chromatographed on histone-agarose as described in Materials and Methods. mRNA decay reaction mixtures were incubated for the indicated times and contained polysomes alone (P), polysomes plus one cell equivalent of unfractionated S130 (PS), polysomes plus one cell equivalent of the histone-agarose-unbound fraction (PU), or polysomes plus or one (1X) or two (2X) cell equivalents of histone-agarose-bound fraction (PB). Total RNA was extracted and analyzed by S1 nuclease protection as for Fig. 1. Annotations are as in Fig. 1.

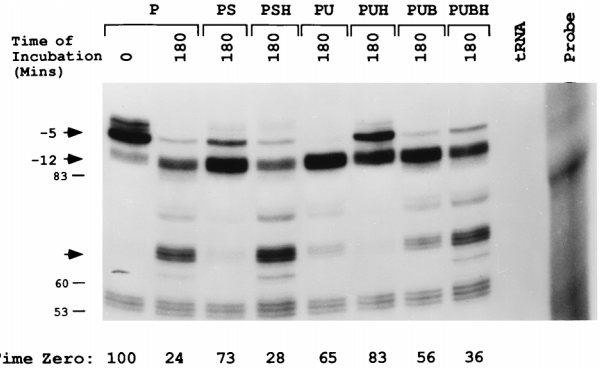


FIG. 3. Identification of destabilizer activity in the bound (B) fraction of a histone-agarose column. S130 was chromatographed on a histone-agarose column, and the unbound (fraction U) and bound (fraction B) material was assayed with polysomes in cell-free decay reactions. RNA was analyzed by S1 nuclease protection as for Fig. 1. Annotations are as in Fig. 1. P, polysomes alone; PS, polysomes plus S130 (1:1 cell equivalency); PSH, polysomes, S130, and histone proteins (1:1 cell equivalency of polysomes and S130); PU, polysomes plus fraction U (1:1 cell equivalency); PUH, polysomes, fraction U, and histones (1:1 cell equivalency of polysomes and fraction U); PUB, polysomes, fraction U, and fraction B (1:1:2 cell equivalency ratio); PUBH, polysomes, fraction U, fraction B, and histones (1:1:2 cell equivalency ratio).

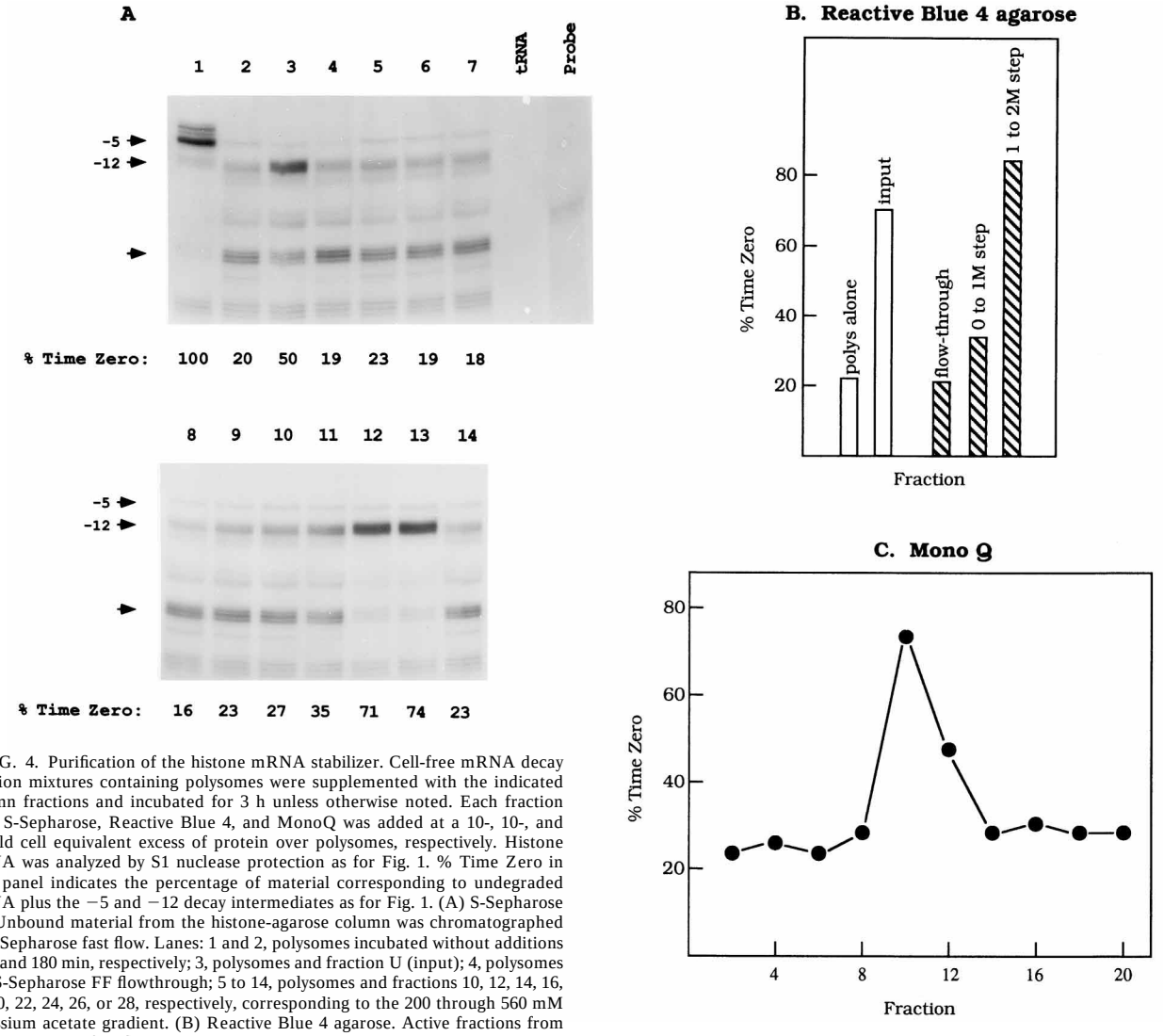


FIG. 4. Purification of the histone mRNA stabilizer. Cell-free mRNA decay reaction mixtures containing polysomes were supplemented with the indicated column fractions and incubated for 3 h unless otherwise noted. Each fraction from S-Sepharose, Reactive Blue 4, and MonoQ was added at a 10-, 10-, and 30-fold cell equivalent excess of protein over polysomes, respectively. Histone mRNA was analyzed by S1 nuclease protection as for Fig. 1. % Time Zero in each panel indicates the percentage of material corresponding to undegraded mRNA plus the −5 and −12 decay intermediates as for Fig. 1. (A) S-Sepharose FF. Unbound material from the histone-agarose column was chromatographed on S-Sepharose fast flow. Lanes: 1 and 2, polysomes incubated without additions for 0 and 180 min, respectively; 3, polysomes and fraction U (input); 4, polysomes and S-Sepharose FF flowthrough; 5 to 14, polysomes and fractions 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28, respectively, corresponding to the 200 through 560 mM potassium acetate gradient. (B) Reactive Blue 4 agarose. Active fractions from the S-Sepharose fast flow column were pooled and chromatographed on Reactive Blue 4 agarose. polys alone, polysomes incubated without any additions; input, polysomes plus pooled active fractions from S-Sepharose; flow-through, polysomes plus unbound material from Reactive Blue 4 agarose; 0 to 1 M step and 1 to 2 M step, polysomes plus the 0 to 1 M or 1 to 2 M potassium acetate step washes, respectively. (C) MonoQ. Material from the 1 to 2 M wash of Reactive Blue 4 agarose was chromatographed on MonoQ. Polysomes were incubated with the flowthrough and with pooled pairs of gradient elution fractions. The fractions shown correspond to those from the 0 to 1 M potassium acetate gradient. The percent time zero values for polysomes alone, polysomes plus input, polysomes plus MonoQ flowthrough material, and polysomes plus the 1 to 2 M potassium acetate wash of MonoQ were 24, 64, 17, and 26, respectively (data not shown).

with high salt. Fraction U slowed histone mRNA degradation and stabilized the −12 product (Fig. 2, compare lanes P, PS, and PU). In other words, fraction U had the same stabilizing effect as did unfractionated S130. Fraction B did not destabilize histone mRNA (compare lane P with PB). Therefore, all the stabilizing activity in the S130 was present in fraction U.

Two experiments confirmed that the histone-agarose column had separated a stabilizer from a histone-activatable destabilizer. (i) When polysomes, S130, and histones were incubated together, the mRNA was destabilized as previously described (Fig. 3, compare lane PS with PSH [52]). However, with polysomes plus fraction U plus histones, there was no destabilization or even modest stabilization (Fig. 3, compare lanes PS, PU, and PUH). Therefore, fraction U contained no destabilizing activity. If it had, histone mRNA would have been destabilized in reactions containing polysomes, fraction U, and histones. (ii) To determine whether the bound fraction (B) contained the destabilizing activity missing from fraction U, a mixture of polysomes plus fractions U and B was incubated with or without histones. Without histones, the mRNA was stabilized approximately twofold (compare lane P with lane PUB). This result confirms that fraction U contains a stabilizer. The mRNA was destabilized when histones were added to the reconstituted mixture (compare lane PUB with lane PUBH). Therefore, fraction B contains the histone-activatable destabilizer.

Purification of the stabilizer activity present in the fraction that did not bind to histone-agarose. We chose to purify the stabilizer activity from fraction U for two reasons. (i) It might up-regulate histone mRNA expression during S phase, when abundant histone protein synthesis is necessary. Therefore, it could play a major role in histone cell cycle regulation. (ii) It would be a valuable reagent for identifying and purifying the destabilizer, because adding purified stabilizer to the reactions

TABLE 1. Purification of the histone mRNA stabilizer

Material	Amt of protein (total units[a])	Sp act[b]	Recovery[c] (%)
Fraction U (histone-agarose)	1,000,000	13,000	
S-Sepharose	13,300		102
Reactive Blue 4	4,200		32
MonoQ[d]	140	700	5

[a] Units are defined in detail in Materials and Methods. Briefly, one unit corresponds to the amount of stabilizer required to change from 68 to 30% the percentage of histone mRNA degraded in 2 h under defined conditions.

[b] Units of stabilizer per 33 μg of protein per 20-μl decay reaction. The specific activity could not be determined for active fractions from S-Sepharose and Reactive Blue 4, because they contained a large excess of carrier protein.

[c] Recovery of stabilizer activity.

[d] Quantitation of pure stabilizer after MonoQ chromatography was carried out by electrophoresing the purified protein and albumin standards in a SDS–12% polyacrylamide gel, staining the gel with Coomassie blue R250, and comparing band intensities by laser-scanning densitometry.

would allow us to assay the destabilizer in the absence of other S130 components. The stabilizer in fraction U prolonged the half-life of the −12 RNA, and this property was used to monitor its purification (Fig. 4; Table 1). S-Sepharose fast-flow chromatography yielded a single stabilizer peak eluting between 440 and 520 mM potassium acetate (Fig. 4A, lanes 12 and 13). No stabilizer was found elsewhere on the column or in the flowthrough, and recovery was approximately 100% (Table 1). Finding activity in a single peak was important, because it excluded the possibility that fraction U contained multiple inhibitors of histone mRNA decay. On Reactive Blue 4 agarose, the stabilizer eluted primarily in the 1 to 2 M potassium acetate wash (Fig. 4B). On MonoQ, the stabilizer again eluted as a single peak between 400 and 500 mM potassium acetate (Fig. 4C, fractions 10 to 12).

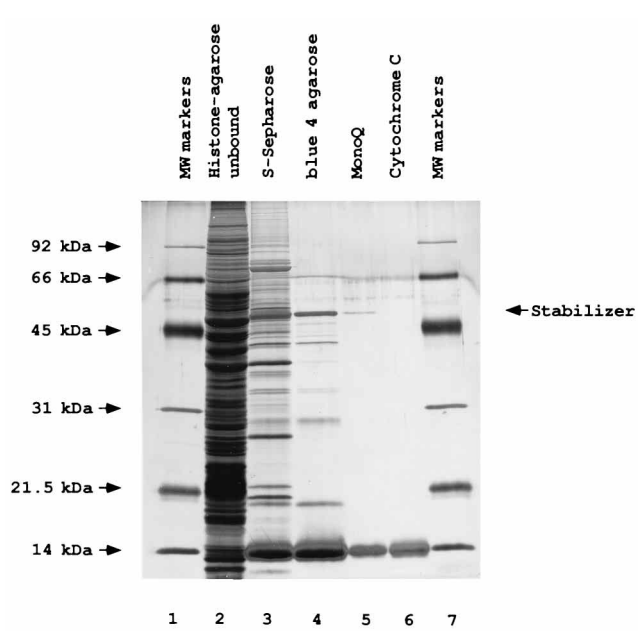


FIG. 5. SDS-polyacrylamide gel electrophoresis analysis of active column fractions. Proteins were electrophoresed in an SDS–12% polyacrylamide gel and visualized by silver staining. Lanes 1 and 7, molecular mass markers, with the mass in kilodaltons noted on the left. Lanes 2 to 5, active fractions from each column (lane 2, 0.005% of the starting material; 3 to 5, 0.2% of the active fractions); the material from S-Sepharose, Reactive Blue 4 agarose, and MonoQ all contained 0.2 mg of cytochrome *c* carrier per ml. Lane 6, cytochrome *c* alone (1 μg). The arrow on the right indicates the band corresponding to purified stabilizer.

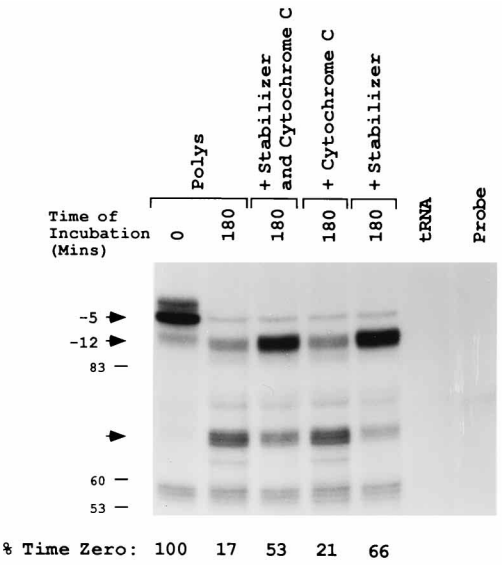


FIG. 6. Effect of pure stabilizer on H4 histone mRNA decay. Cell-free mRNA decay reaction mixtures contained polysomes (Polys), polysomes plus 0.12 μg of stabilizer plus 0.32 μg of cytochrome *c* (+ Stabilizer and Cytochrome C), polysomes plus 0.32 μg of cytochrome *c* (+ Cytochrome C), or polysomes plus 0.12 μg of stabilizer (+ Stabilizer). The (+ Stabilizer) lane contained no cytochrome *c*. Histone mRNA decay was analyzed as for Fig. 1. Annotations are as in Fig. 1.

One ~52-kDa band was detected in a silver-stained SDS-polyacrylamide gel of MonoQ-purified material (Fig. 5, lane 5). (Other bands in this lane were from the cytochrome *c* carrier). Cytochrome *c* itself had no effect on histone mRNA decay, and the ~52-kDa protein purified without carrier was active (Fig. 6). We conclude that the ~52-kDa protein is the stabilizer.

The stabilizer is the La protein. Since the N terminus of the stabilizer was blocked, the purified protein was cleaved with trypsin, and internal peptide fragments were purified and sequenced. Two peptides, WIDFVR and LHILFSNHGEIK, were identical to sequences found in the human La protein. Moreover, stabilizer purified to homogeneity reacted in a Western blot with monoclonal antibody against human La (Fig. 7). La was easily detected in the S130, which was the starting material from which it was purified. Some La was also detected in polysomes, which is consistent with the binding of La to 18S rRNA (50) and to cell mRNAs (38). La in the S130 appeared to migrate slightly faster than purified La. We have not investigated this difference but suspect that it might have resulted from modest overloading of the S130 lane.

To confirm that La was responsible for histone mRNA stabilization in vitro, polysomes and S130 were incubated in mRNA decay reactions in the presence of anti-La antiserum or preimmune serum. Anti-La blocked stabilization of the −12 RNA (Fig. 8, compare lane PS with lane PS + α-La). Preimmune serum had no effect. These data support the purification and sequencing results and implicate La as a stabilizer of histone mRNA.

Specificity of La for stabilizing histone mRNA. Cell cycle-regulated histone mRNAs differ in several respects from most other mRNAs. They are transcribed from intronless genes, lack poly(A), and are expressed abundantly only during S phase. Therefore, it is not surprising that specific factors like

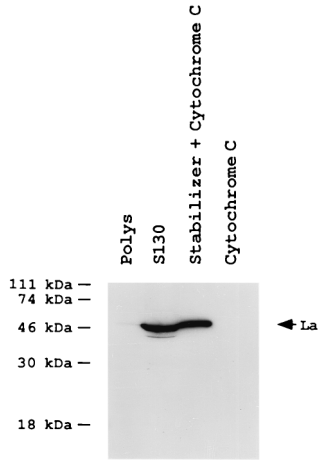


FIG. 7. Western blot analysis of polysomes, S130, and pure stabilizer with anti-La monoclonal antibody. Polysomes (Polys) and S130 from 10^6 cells, a mixture of 0.4 μg of purified stabilizer plus 1 μg of carrier cytochrome *c*, or 1 μg of cytochrome *c* alone was electrophoresed in an SDS–12% polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane and probed with a 1:50 dilution of anti-La monoclonal antibody SW5 (49). La was visualized as described in Materials and Methods. Positions of molecular mass markers are indicated on the left. La is indicated by the arrow on the right.

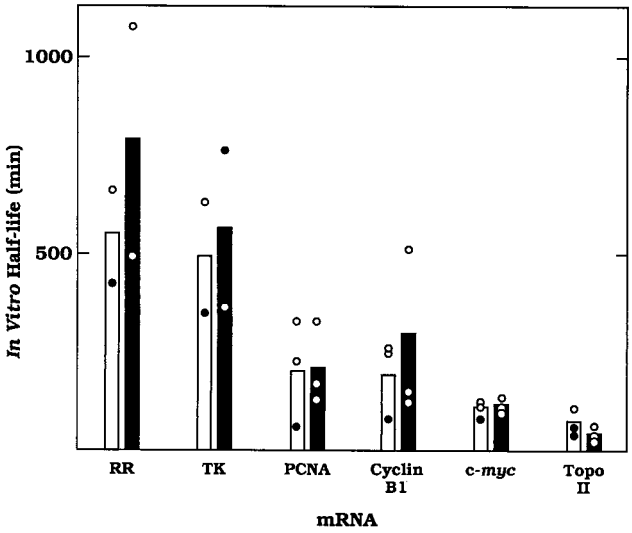


FIG. 9. Lack of effect of La on the decay of other mRNAs. Cell-free mRNA decay reaction mixtures were preincubated at 4°C for 30 min and then incubated at 37°C for various times. Reaction mixtures contained polysomes alone or polysomes plus 0.12 μg of purified La. Total RNA was extracted and blotted, and the mRNAs of interest were visualized with [^{32}P]DNA probes. Each blot was also hybridized with a 5′-end-labeled oligodeoxynucleotide for 28S rRNA. Radiolabeled bands were quantitated with a PhosphorImager. Half-lives were determined after correcting for loading differences by using the 28S rRNA value as a standard. The results summarize experiments with different preparations of polysomes, and each half-life value is indicated by a single point. Bars represent the average of two or three measurements. Half-lives longer than 3 h (for ribonucleotide reductase, thymidine kinase, proliferating-cell nuclear antigen, and cyclin B1) are extrapolations, because in vitro decay rates are not linear after several hours (55a). Open bars, polysomes alone; solid bars, polysomes plus La. Abbreviations: RR, ribonucleotide reductase subunit 1; TK, thymidine kinase; PCNA proliferating-cell nuclear antigen; Topo II, topoisomerase II.

La and the destabilizer regulate histone mRNA. On the other hand, the in vitro mRNA decay system used in these studies does not mimic the intracellular environment in all respects. In particular, polysomal mRNAs are not translated in this system. For these and other reasons, it was essential to assess the specificity of purified La by determining whether it affected other mRNAs. mRNA decay reactions were incubated in the presence or absence of purified La, and the half-lives of six mRNAs were determined. Five of the six are cell cycle regulated. However, none of the six was affected by La to the same extent as histone, and none was stabilized by even twofold (Fig. 9). The half-lives of the longer-lived mRNAs for ribonucleotide reductase, thymidine kinase, proliferating-cell nuclear antigen, and cyclin B1 were more variable than those of the shorter-lived mRNAs. This variability probably reflects the fact that the half-lives of the stabler mRNAs were derived by extrapolation, which was necessary because mRNA decay rates do not remain linear after several hours of incubation (55a). In spite of the variability, it is important to note that La did not influence any of the tested mRNAs by even twofold, while it consistently stabilized histone mRNA by threefold or more. Therefore, La is not a stabilizer of all cell cycle-regulated mRNAs.

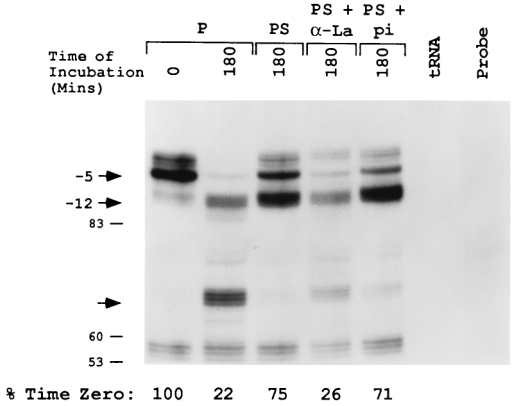


FIG. 8. Effect of human anti-La serum on S130-dependent stabilization of H4 histone mRNA. Cell-free mRNA decay reaction mixtures contained polysomes alone (P) or polysomes plus S130 (PS). Where noted, "Gore" anti-La antiserum (α-La [10]) or preimmune (pi) serum was added to a final protein concentration of 200 μg per reaction mixture. The reaction mixtures were incubated and analyzed as for Fig. 1. Annotations are as in Fig. 1.

DISCUSSION

A histone mRNA-stabilizing activity was detected in a postribosomal supernatant fraction (S130) by comparing histone mRNA decay in cell-free reaction mixtures containing polysomes alone or polysomes plus S130. The stabilizer was then separated from a destabilizer by chromatography on histone-agarose. The destabilizer is normally dormant but is activated by free histones. When activated, it overcomes the stabilizer and accelerates histone mRNA degradation (Fig. 3) (39, 52). A single stabilizing activity was observed when all fractions of an S-Sepharose column were assayed, showing that the stabilizer is a discrete activity with a specific mRNA target (Fig. 4A). Subsequent purification and characterization revealed the stabilizer to be La, a RNA-binding protein (9, 71). Purified La stabilized histone mRNA but not six other mRNAs, five of which are cell cycle regulated (Fig. 9).

Our studies do not address the mechanism of action of La. We do not know if La is actually bound to histone mRNA or acts indirectly, although other in vitro studies suggest that La can bind to strong translation initiation regions of mRNAs (38). However, La does not appear to affect the half-lives of

most other mRNAs. Therefore, one or more special features of histone mRNA probably make it a target of La-mediated regulation. One such feature is the 3′-terminal stem-loop. It is unique to cell cycle-regulated histone mRNAs, and it associates with a stem-loop-binding protein whose action is thought to influence the intranuclear processing and cytoplasmic stability of the mRNA (34, 40, 44, 72, 73, 75). The stem-loop region is particularly relevant to histone mRNA degradation for two reasons. First, the mRNA is degraded in the 3′-to-5′ direction in cells and cell extracts (39, 58). Second, the 3′ termini of the earliest decay intermediates are located within the stem-loop (58).

An important feature of La is its ability to bind to and affect the function of so many RNAs in different ways. It is found in mammalian, insect, and yeast cells and is located in both in the nucleus and cytoplasm (12, 20, 30, 49, 66, 78). In the nucleus, it associates with the U-rich 3′ termini of newly synthesized RNA polymerase III transcripts, facilitates transcriptional termination and transcript release from the DNA (18, 19, 33), and even promotes the initiation of polymerase III transcription (32). It also binds to small RNAs in some virus-infected cells (28, 48, 70). Three cytoplasmic functions have been described for La. (i) It facilitates the translation of poliovirus RNA by binding to the internal ribosome entry site, stimulating initiation at the correct start codon, and suppressing it at incorrect ones (41). It binds to the TAR stem-loop of human immunodeficiency virus type 1 RNA and facilitates the translation of downstream open reading frames (10, 69). It might even play a role in translation initiation in uninfected cells, since it interacts in vitro with the translation start site (38). (ii) It facilitates Sindbis virus RNA replication (48). (iii) It stabilizes histone mRNA. How a single protein effects such apparently diverse processes is unclear. It is clear that La binds to a variety of RNA structures and changes its conformation in different ways depending on the RNA to which it binds (55). Therefore, it seems to be highly adaptable for a variety of RNA interactions. Other mammalian and prokaryotic RNA-binding proteins are also multifunctional and protect mRNAs from degradation (5, 8, 17, 24, 37).

Regardless of the mechanism of action of La, our findings implicate La in a process perhaps essential for producing a full complement of histones during S phase. Since histone mRNA lacks poly(A) and is inherently relatively unstable, we suggest that it is exposed to mRNase attack throughout S. The mRNase nibbles at the 3′ terminus but is blocked by La from advancing too rapidly toward the 5′ end and destroying the coding region. La-mediated mRNA stabilization would prolong histone translation and facilitate the production of sufficient histones to coat all the nucleosomes. At the end of S, histone proteins accumulate in the cytoplasm and activate the destabilizer, which neutralizes La and accelerates histone mRNA decay. The post-S autoregulation of histone mRNA thus ensures that potentially toxic histones do not accumulate during M and G_1.

We do not know if La binds to histone mRNA or to any other mRNA in cells. However, La does bind in vitro to RNAs whose 3′ termini contain three or four U residues (18, 19, 36). This finding, coupled with our data on La-induced mRNA stabilization, suggests that La might stabilize mRNAs whose 3′-untranslated regions contain U-rich segments. There are a number of such mRNAs, including thrombospondin, c-*fos*, and cytoplasmic β-actin (11, 23, 53; see also references 26, 42, and 77). If these mRNAs are degraded in a 3′-to-5′ direction, decay intermediates with oligo(U) at their 3′ termini might be generated. These termini could function as La-binding sites, and the La-oligo(U) complex could retard the mRNase. There is some precedent for such a mechanism, since α-globin mRNA is stabilized by proteins that bind to a C-rich segment of its 3′-untranslated region (74).

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