Expression of the lactate dehydrogenase A subunit (LDH-A) gene can be controlled by transcriptional as well as posttranscriptional mechanisms. In rat C6 glioma cells, LDH-A mRNA is stabilized by activation and synergistic interaction of protein kinases A and C. In the present study, we aimed to identify the sequence domain which determines and regulates mRNA stability/instability by protein kinase A and focused our attention on the 3′-untranslated region (3′-UTR) of LDH-A mRNA. We have constructed various chimeric globin/lactate dehydrogenase (ldh) genes linked to the c-fos promoter and stably transfected them into rat C6 glioma cells. After their transfection, we determined the half-life of transcribed chimeric globin/ldh mRNAs. The results showed that at least three sequence domains within the LDH-A 3′-UTR consisting of nucleotides 1286–1351, 1453–1471, and 1471–1502 are responsible for the relatively rapid rate of LDH-A mRNA turnover in the cytoplasm. Whereas chimeric globin/ldh mRNAs containing the base sequences 1286–1351 and 1453–1471 were not stabilized by (S9)-cAMPS, an activator of protein kinase A, instability caused by the 1471–1502 domain was significantly reversed. Additional deletion and mutational analyses demonstrated that the 3′-UTR fragment consisting of the 22 bases 1478–1499 is a critical determinant for the (S9)-cAMPS-mediated LDH-A mRNA stabilizing activity. Because of its functional characteristics, we named the 22-base region “cAMP-stabilizing region.”

Analysis of the LDH1 isoenzyme patterns in various cell types under a variety of physiologic conditions suggests complex regulatory mechanisms that determine specific isoenzyme expression (1–7). The LDH-A subunit, for instance, is subject to regulation by a number of different effector agents such as estrogen (3, 8), epidermal growth factor (5), catecholamines (4, 9), phorbol ester (7), and c-Myc (10), which change the isoenzyme pattern almost exclusively in favor of the LDH-5 (A4) isoenzyme. The functional importance of these LDH isoenzyme shifts is generally attributed to a need for increased A subunit-isoenzyme. The functional importance of these LDH isoenzyme patterns almost exclusively in favor of the LDH-5 (A4) LDH isoenzyme is subject to regulation by agents that activate second messenger signal pathways.

It has recently been demonstrated that activators of protein kinases A and C are important effectors of mRNA stability regulation in a number of gene systems (14). Although it is known that increased levels of either cAMP or phorbol ester are sufficient signals for increased LDH-A mRNA stability, the molecular mechanisms mediating the effects of cAMP or phorbol ester on mRNA stability have not been defined in detail. Based on our previous data (4, 12), we suggested that sequences within the non-coding regions of LDH-A mRNA together with protein kinase-regulated RNA-binding phosphoprotein(s) may play a pivotal role in determining the basal and regulated stability of mRNA (12, 22).

In the present study, we have chosen to identify putative cis-regulatory domains within the 3′-UTR of LDH-A mRNA that are involved in protein kinases A-mediated mRNA stability regulation. Our initial approach was to express transfected chimeric β-globin/ldh 3′-UTR constructs and to evaluate the functional effects of protein kinase A on chimeric mRNA stability. Furthermore, applying ribonuclease protection assay to
determine the half-life of truncated and mutated fragments of LDH-A 3'-UTR, we systematically analyzed the 3'-UTR for the presence of (a) sequence domain(s) that cause mRNA instability, and (b) stability-regulatory domain(s) whose activity is modulated by protein kinase A. Our experiments demonstrate that several 3'-UTR fragments evoked marked instability of the otherwise relatively stable β-globin mRNA. Most importantly, we were able to identify a uridine-rich cAMP-stabilizing region (CSR) responsible for regulating the rate of LDH-A mRNA turnover in response to activators of protein kinase A and the phosphatase inhibitor okadaic acid.

EXPERIMENTAL PROCEDURES

Materials—Nucleic acid-modifying enzymes, acrylamide, nucleoside triphosphates were purchased from Boehringer Mannheim. Radioisotopes were purchased from NEN Life Science Products. Other reagents were of molecular biology grade and purchased from Sigma. Cell culture products were purchased from Life Technologies, Inc. (S$_4$)-Adenosine 3',5'-cyclic monophosphorothioate (S$_4$)-cAMPS and (R$_4$)-adenosine 3',5'-cyclic monophosphorothioate (R$_4$)-cAMPS were from BIOLOG Life Science Institute.

Synthetic Oligonucleotides—Synthesis and processing of synthetic DNA oligonucleotides and their ligation into the respective plasmid vectors were performed as described previously (23).

Cell Culture—Rat C6 glioma cells (ATCC CCL 107) were maintained as monolayers in Ham's F-10 nutrient medium supplemented with 10% dialyzed fetal calf serum, 50 units/ml penicillin, and 50 μg of streptomycin as described by us (9). All experiments were carried out at about 90% confluence, and serum was withdrawn 16–18 h prior to addition of various agents.

Plasmids—A rat fibroblast LDH-A cDNA clone (pLDH-2), provided by Dr. Richard Breathnach, contains the full-length 1609-bp cDNA insert. The mRNA has a 103-nucleotide 5'-nontranslated region and a 510-nucleotide 3'-untranslated region, corresponding to nucleotides 1103 through 1613 (5). The 3'-UTR contains the classic polyadenylation signal AATAAA 18 nucleotides before the poly(A) sequence. A HindIII/BamHI fragment containing the entire LDH-A 3'-UTR (with 28-bp 5'-coding sequence and 100-bp pLDH-2 vector sequence) was inserted into pGem3Zf(+) at the BamHI site resulting in plasmid pLDH-5. Plasmid pLDH-6 was constructed to retain the complete 3'-UTR but eliminate the 28-bp LDH-A coding and 100-bp pLDH-2 vector sequences contained in pLDH-5. Using oligonucleotide primers, the complete 510-bases of the 3'-UTR of LDH-A with 5' BamHI and 3' HindIII sites was amplified by polymerase chain reaction (PCR) from pLDH-5. The fragment was cloned into the BamHI/HindIII sites of pBluescript II KS+ (Stratagene).

The rabbit β-globin expression vector pRe/FBB was constructed by Dr. D. Chagnovich (Northwestern University) in two steps from plasmids pRe/CMV (Invitrogen) and pBBB (kindly provided by Dr. M. E. Greenberg). To the purpose, pRe/CMV was linearized with BglII and blunt-ended with T4 DNA polymerase. After insertion of a deamer containing a SacII restriction site, the resulting vector was cut with SacII and HindIII to excise the cytomegalovirus promoter and to serve as acceptor for the modified pBBB. For this modification a SacII-Nru I fragment was excised from pBBB and replaced with HindIII linkers. After restriction with SacII, pRe/FBB was created by linking modified pBBB with the modified SacII-HindIII fragment of pRe/CMV. Plasmid pRe/FBB encodes a transcription unit consisting of β-globin coding region flanked by the β-globin 5'- and 3'-untranslated regions fused to the c-fos promoter. The sequence and correct orientation of all inserts were verified by restriction and DNA sequence analyses. Sequencing was carried out in both directions by the dideoxynucleotide chain terminator method with specific synthetic oligonucleotides as primers.

Stable Transformation with Expression Vectors and Selection of G418-resistant Clones—One day prior to transfection, cell cultures were prepared by seeding 1 x 10$^5$ cells/60-mm plate in medium containing 10% fetal calf serum. Each plate was treated with 100 μg of Lipofectin in 2 ml of Opti-MEM I for 20 min after which 10 μg of supercoiled plasmid DNA were added. After 6 h, the Lipofectin solution was replaced with 3 ml of DMEM supplemented with 2% fetal calf serum. Two days following transfection, cells were trypsinized and replated at several dilutions between 1:5 and 1:10 in selective medium containing 0.5 mg/ml G418 (Geneticin). Cells were fed with selective medium every third day until resistant colonies were clearly visible (after about 2 weeks). Individual drug-resistant colonies were subcloned and expanded under selection conditions.

RESULTS

LDH-A 3'-UTR Is Responsible for mRNA Instability—It is known that the presence of AU-rich regions in the 3'-UTR may function as destabilizing elements in several mRNAs. Sequence analysis of the LDH-A 3'-UTR identifies a 99-nucleotide stretch (nucleotides 1450–1549), which is relatively AU-rich when compared with the overall nucleotide composition of the 3'-UTR. Whether or not this AU-rich region contains a site(s) that determines LHDA-A mRNA stabilizing/destabilizing activity and can additionally be modulated through protein kinase signal pathways has so far not been determined. As a first step to experimentally identify stability/instability elements in the 3'-UTR of LDH-A mRNA, we replaced the 3'-UTR of β-globin mRNA in plasmid pRe/FBB with the entire LDH-A 3'-UTR and then determined the rate of decay and half-life of the chimeric globin/ldh mRNA under various experimental conditions. By choosing an expression vector (pRe/FBB) with a serum-inducible c-fos promoter (15, 27, 28), we also avoided artifacts that potentially occur when commonly used transcriptional inhibitors such as actinomycin D (29, 30) are used to stop ongoing transcription. To perform the decay studies, the pRe/FBB/ldh construct was introduced into rat C6 glioma cells by stable transfection. G418-resistant colonies were pooled to ensure a heterogeneity of integration sites. Quantitative ribonuclease protection assays for each chimeric vector were performed to measure mRNA half-lives. After serum-starving the transfected cells for 25–30 h, pulse induction of the chimeric globin/ldh gene under the control of the c-fos promoter was done by addition of fetal calf serum. Total cytoplasmic RNA was isolated at subsequent time points and ribonuclease protection assays were done. As shown in Fig. 1 (panel A, Lane a, 650 β-globin), wild-type β-globin mRNA was remarkably stable and persisted in the cytoplasm with a half-life of about 21 h (extrapolated from Fig. 1, panel B), similar to data obtained by others (28, 31–33). In stark contrast, the chimeric β-globin/ldh mRNA (panel A, Lane Control) decayed at a much faster rate ($t_{1/2}$ ~ 70 min) (Fig. 1, panel B), similar to the rapid rate of decay of wild-type LDH-A mRNA ($t_{1/2}$ ~ 55 min) in glioma (4, 12). For each chimeric vector the decay data consistently obeyed first-order kinetics. Since the fetal calf serum levels during the copy number of integrated globin genes after transfection, we analyzed the copy number of integrated globin genes by Southern blot analysis (not shown). We found that the average number of integrated globin copies is nearly the same for all transfected cells.

By treating the transfected cells with the protein kinase A agonist, (S$_4$)-cAMPS, we found that the activated protein ki-
nase had the ability to alter the stability of chimeric globin/ldh mRNA. \((S_p)-cAmps\) significantly prolonged the half-life of hybrid globin/ldh mRNA (panel A, lane \(Sp\)-cAMPS) but not that of wild-type globin mRNA (not shown). For instance, the half-life of globin/ldh mRNA increased from about 70 min in untreated cells to about 8 h in \((S_p)-cAmps\)-treated cells (see panel B).

Inasmuch as the above data suggested the involvement of protein kinase A and, hence, protein phosphorylation, we sought additional insight into the significance of potential phosphorylation events by preventing protein phosphorylation through the use of inhibitors of protein kinase A. We used the selective protein kinase A antagonist \((R_p)-cAMPS\) to prevent protein kinase activation. The results summarized in Table I show that \((R_p)-cAMPS\) blocks protein kinase A-mediated stabilization of globin/ldh mRNA. Furthermore, we used okadaic acid, an inhibitor of protein phosphatases-1 and -2A (34), to shift the overall balance of phospho-/dephosphoproteins in favor of the phosphorylated proteins. Exposure of transfected cells to okadaic acid \((20 \text{ nM})\) resulted in a much slower decay and a markedly enhanced stability of globin/ldh mRNA (see Fig. 2).

Thus, the studies show that the half-life of chimeric globin/ldh mRNA and its regulation are similar, if not identical, to that of wild-type LDH-A mRNA and that the 3′-UTR contains all elements needed to (a) destabilize LDH-A mRNA and (b) to convey protein kinase-mediated stability to globin mRNA. We conclude that regulation of LDH-A mRNA stability is an inherent function of its 3′-UTR and that it is not, or is only to some minor degree, affected by other regions of the mRNA (such as coding regions and 5′-UTR).

**LDH 3′-UTR Contains at Least Three Determinants of Instability**—Based on above data, we proceeded to identify putative regions within the LDH-A 3′-UTR that are \((a)\) instrumental in determining instability of LDH-A mRNA and \((b)\) responsible for the protein kinase-mediated stabilization of the mRNA. To that purpose, we generated a series of systematically truncated 3′-UTR fragments that were inserted into the unique \(5′\)-UTR fragment. Cells were stably transfected with pRc/FBB in which the entire \(5′\)-UTR was replaced with the entire LDH 3′-UTR fragment. Cells were treated for 6 h with the indicated concentrations of agents. After addition of serum, RNA was isolated at various time points up to 12 h. Globin/ldh mRNA decay was assessed by ribonuclease protection assay, and half-lives were determined as described under “Experimental Procedures.” Results are expressed as mean and S.E. of four separate experiments.

### Table I

| Treatment                  | Half-life (t\(_{1/2}\)) |
|----------------------------|------------------------|
| None                       | 69 ± 4 min             |
| \((S_p)-cAMPS\) (500 \(\mu\text{M}\)) | 8.7 ± 1.25 h           |
| \((R_p)-cAMPS\) (500 \(\mu\text{M}\)) | 78 ± 4 min             |

**Fig. 1.** Decay of chimeric globin/ldh mRNA as a function of time. Rat C6 glioma cells were stably transfected with pRc/FBB in which the entire \(\beta\)-globin 3′-UTR had been replaced with the LDH 3′-UTR fragment. Cells were treated for 6 h with 500 \(\mu\text{M}\) \((S_p)-cAMPS\). After addition of serum, RNA was isolated at the indicated time points. Decay was assayed by ribonuclease protection assay. For details, see “Experimental Procedures.” Panel A, autoradiographs showing the decay of chimeric globin/ldh mRNA before (Control) and after \((S_p)-cAMPS\) treatment \((Sp-cAMPS)\). Also shown is the decay of wild-type \(\beta\)-globin mRNA and glyceraldehyde-3-phosphate dehydrogenase mRNA. Panel B, radioactivity was quantified using a radioanalytic imaging scanner. The results were plotted using nonlinear regression analysis with the InPlot program. Values are expressed as percent of zero time. The experiments were repeated four times with similar results. ●, control; ■, \((S_p)-cAMPS\)-treated cells; ▲, wild-type \(\beta\)-globin mRNA.

**Fig. 2.** Decay of chimeric \(\beta\)-globin/ldh mRNA in untreated and okadaic acid-treated rat C6 glioma cells. Cells were stably transfected and treated with serum as described in legend of Fig. 1. RNA was isolated at the indicated time points. Panel A, autoradiographs showing the decay of chimeric globin/ldh mRNA before (Control) and after okadaic acid treatment. Panel B, radioactivity was quantified as described in legend of Fig. 1. ●, control; ▲, okadaic acid-treated. For experimental conditions see Experimental Procedures.
Cyclic AMP-regulated mRNA Stabilizing Region

Effect of (Sp)-cAMPS on the half-life of chimeric globin/ldh mRNAs

Rat C6 glioma cells were stably transfected with pRc/FB in which the listed fragments of LDH-A 3'-UTR (with 5' and 3' BglII ends) had been inserted into the BglII site of pRc/FBB (see Fig. 3). Cells were treated in serum-free medium for 6 h with 0.5 mM (Sp)-cAMPS. After addition of serum, mRNA was isolated at various time points up to 12 h. Globin/ldh mRNA decay was assessed by ribonuclease protection assay, and half-lives were determined as described under “Experimental Procedures.” Results are expressed as mean and S.E. of four separate experiments.

| Fragment inserted (base no.) | Half-life (h) |
|-----------------------------|---------------|
| Control | (Sp)-cAMPS | (Sp)-cAMPS/Control |
| 1103–1610 | 1.2 ± 0.6 | 8.3 ± 0.9 | 7.1 |
| 1286–1351 | 3.9 ± 0.5 | 3.1 ± 0.7 | 0.8 |
| 1453–1470 | 2.9 ± 0.9 | 3.6 ± 1.2 | 1.2 |
| 1453–1527 | 4.5 ± 0.3 | 15.6 ± 2.1 | 3.5 |
| 1463–1502 | 4.1 ± 0.6 | 17.5 ± 2.1 | 4.3 |
| 1463–1527 | 3.7 ± 0.7 | 16.9 ± 2.4 | 4.6 |
| 1471–1502 | 3.9 ± 0.8 | 17.8 ± 2.5 | 4.6 |

Deletion of Fragment 1478–1502 Causes Loss of (Sp)-cAMP Responsiveness of LDH-A 3’-UTR—To expand and confirm the above data, we carried out a stability analysis of LDH-A 3’-UTR in which partial base sequences had systematically been deleted from the 3’-UTR. Globin 3’-UTR in pRc/FB was removed by BglII/HindIII digestion and replaced with truncated LDH-A 3’-UTR fragments. After stable transfection of the vectors into rat C6 glioma cells, the vectors were transcribed, and the decay characteristics of the chimeric globin/ldh mRNAs were analyzed. The half-lives determined by this analysis are summarized in Table III. While deletion of various fragments had little effect on destabilization and (Sp)-cAMP responsive-ness, deletion of fragment 1453–1527 and the even shorter fragment 1478–1502 resulted in increased stability and loss of (Sp)-cAMP responsiveness confirming that a cAMP-responsive instability/stability element is present in fragment 1478–1502.

Bases 1478–1499 Are Critical for (Sp)-cAMP-mediated LDH-A mRNA Stabilizing Activity—Having shown that the 1478–1502 nucleotide sequence was required to achieve (Sp)-cAMP-mediated mRNA stabilization, we further characterized the region by mutational analysis. We inserted bases 1478–1504 in the correct and reverse orientation into the BglII site of pRc/FBB and analyzed the decay characteristics. Insertion in the correct orientation (1478–1504wt) caused instability of the chimeric mRNA and stabilization by (Sp)-cAMPs (Table IV), while positioning in the reverse orientation (1478–1504rev) had no effect on stability or (Sp)-cAMP responsiveness, indicating that the destabilizing and regulatory effects of CSR required a specific polarity of the base sequence.

Several mutational changes throughout the 27-base 1478–1504 fragment led to a further definition of the active (Sp)-cAMPs-responsive base region. Introduction of systematic linker scanning mutations from 1478 through 1502 (mut1 through mut4) and also randomly-placed base mutations (mut5) did not abolish mRNA destabilization (Table IV). However, the (Sp)-cAMPs stabilizing effect was completely lost in mut1, -2, -3, and -5. In contrast, the mutations at 1500–1502 had no effect on (Sp)-cAMPs-mediated stabilization.

DISCUSSION

During recent years, we have provided evidence indicating a transcriptional as well as posttranscriptional regulation of the LDH-A subunit gene. Clues to mechanisms underlying this dual mode of control were provided by our previous identifica-
After replacement of the entire instability as well as the functional effects of protein kinase A.

### TABLE III

| Fragment deleted (base no.) | Half-life (h) Control | (S<sub>S</sub>)<sub>-cAMPS</sub> | (S<sub>S</sub>)<sub>-cAMPS</sub>/control |
|-----------------------------|-----------------------|-------------------------------|---------------------------------------|
| 1286–1351                   | 2.7 ± 0.5             | 16.6 ± 2.1                    | 4.5                                   |
| 1340–1410                   | 2.9 ± 0.3             | 15.8 ± 1.9                    | 5.4                                   |
| 1404–1462                   | 3.9 ± 0.8             | 16.6 ± 2.2                    | 4.2                                   |
| 1453–1527                   | 9.8 ± 2.9             | 9.7 ± 2.3                     | 1.0                                   |
| 1478–1502                   | 9.9 ± 1.9             | 9.7 ± 1.5                     | 0.9                                   |
| 1527–1590                   | 3.6 ± 0.7             | 15.6 ± 2.1                    | 4.3                                   |

### TABLE IV

| Fragment inserted (base no.) | Half-life (h) Control | (S<sub>S</sub>)<sub>-cAMPS</sub> | (S<sub>S</sub>)<sub>-cAMPS</sub>/control |
|-----------------------------|-----------------------|-------------------------------|---------------------------------------|
| 5<sup>`</sup>′ ATATTTTCTGATATTAAATGTGCTGTA 3<sup>`</sup> ′ wt 5<sup>`</sup>′ TCTGGGCTATTTAAAATGTGCTGTA 3<sup>`</sup> ′ mut1 5<sup>`</sup>′ ATATTTTCTGATTTAAATGTGCTGTA 3<sup>`</sup> ′ mut2 5<sup>`</sup>′ ATATTTTCTGATTTgacaATGTGCTGTA 3<sup>`</sup> ′ mut3 5<sup>`</sup>′ ATATTTTCTGATTTAAATGTGCTGTA 3<sup>`</sup> ′ mut4 5<sup>`</sup>′ ATATTTTCTGATTTAAATGTGCTGTA 3<sup>`</sup> ′ mut5 |
| 1478–1504wt                 | 4.3 ± 0.4             | 10.5 ± 0.9                    | 2.4                                   |
| 1478–1504rev                | 14.7 ± 2.6            | 15.5 ± 1.8                    | 1.1                                   |
| 1478–1504mut1               | 5.1 ± 0.9             | 4.9 ± 1.1                     | 0.9                                   |
| 1478–1504mut2               | 4.5 ± 0.8             | 5.6 ± 0.6                     | 1.2                                   |
| 1478–1504mut3               | 4.1 ± 1.1             | 5.5 ± 0.7                     | 1.3                                   |
| 1478–1504mut4               | 3.7 ± 0.5             | 11.1 ± 1.9                    | 3.0                                   |
| 1478–1504mut5               | 3.9 ± 0.7             | 4.8 ± 0.8                     | 1.2                                   |

### cis-Acting Elements

To localize the sequence domains within the 3′-UTR responsible for instability and for stabilization, we have used LDH-A 3′-UTR from which small fragments had been systematically deleted as well as truncated and mutated 3′-UTR fragments to construct chimeric globin/ldh vectors. After their transfection and transcription, we determined the decay characteristics of the resulting chimeric globin/ldh mRNAs. This experimental approach allowed us to identify two types of destabilizing sequences within the LDH 3′-UTR: (i) two regions at base positions 1286–1351 and 1453–1471, which do not respond to (S<sub>S</sub>)<sub>-cAMPS</sub> stimulation; and (ii) an U-rich (53%) 22-base sequence at 1478–1499, -AUAAUUUGUAUUAAUUGU-, whose presence in the 3′-UTR destabilizes the message and responds to protein kinase A activation with a marked mRNA stabilization. Based on its function, we have named the 22-base sequence “cAMP-stabilizing region” (CSR). The unregulated destabilizing regions (bases 1286–1351 and 1453–1471) have a uridine content of 24, respectively 40%. Although they markedly reduce the half-life of normally long-lived globin mRNA when inserted as truncated fragments into globin mRNA, they make only a minor destabilizing contribution in globin/ldh mRNA containing the complete LDH-A 3′-UTR from which the CSR had been deleted (see for instance Table IV). Thus, the most significant results of the present work consist of identification of a region (bases 1478–1499) with a twofold function: (a) it acts as an U-rich instability element, and (b) it functions specifically as a dominant stabilizer of LDH-A mRNA half-life in response to activation of the protein kinase A signal transduction pathway.

### cis-Acting Elements

One example is the occurrence of a class of short-lived mRNAs that share AU-rich motifs in their 3′-UTRs (14). Although a consensus sequence of -AUUUA- was identified as the potential signal for instability (15), it is known that the -AUUUA- sequence or multimers thereof may not be the only signals for destabilization of mRNAs. The CSR is positioned within an AU-rich environment. Although we cannot assess the contribution of the nucleotide environment to the specificity of CSR action at this time, it is conceivable that it may determine a specific secondary structure and RNA conformation necessary for interaction with trans-regulatory proteins.

The present research extents our knowledge of cognate 3′-UTR sequences involved in agonist-mediated mRNA stabilization. We know that the turnover rates of a number of mRNA species can be modulated by different biological signals. For instance, treatment of cells with phorbol ester (12, 36–38), antibodies to cell surface proteins (39), serum (40), steroid and thyroid hormones (41–45), and cAMP-generating agonists (4, 46–48) can modulate the half-lives of unstable messages. It is generally accepted that the regulation of mRNA turnover depends on a variety of specific cis-acting sequences and trans-acting factors (49). The trigger event for mRNA decay consists of several steps involving poly(A) shortening, impaired translation, and nucleolytic cleavage. Additional complexity of the decay mechanisms is added by the nature of the primary and secondary structure of the stabilizing/destabilizing 3′-UTR element, the destabilizing nucleolytic enzymes, and by trans-acting factors that may modulate the interaction of nucleases with the mRNA.

In another paper (22), we have described a complementary experimental approach to the identification of cis-regulatory sequences in the LDH-A 3′-UTR by searching for putative trans-regulatory components, e.g., proteins that bind to the CSR and may be involved in regulating LDH-A mRNA stability. We were able to identify four CSR-binding proteins whose binding
activity was up-regulated after cAMP-mediated activation of protein kinase A, implying a correlation between phosphorylative and functional modification of nucleolytic enzymes. However, this scenario makes several assumptions: (a) the nucleolytic enzyme(s) is LDH mRNA sequence-specific, (b) the nuclease(s) is phosphorylated by protein kinase A leading to its deactivation, (c) the nuclease(s) may be specifically recognized by a component(s) of the activated signal transduction systems and as a result of the interaction be functionally modified. Although we believe this scenario to be unlikely, it should be noted that Bandyopadhyay and co-workers (50) have observed that polysome preparations may contain a nuclease activity that is subject to inhibition by a soluble cytoplasmic factor. Thus, the possibility that the activity and specificity of nucleolytic enzymes may be regulated by protein kinase-modified factors needs further study.

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