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Multiple Instability-Regulating Sites in the 3′ Untranslated Region of the Urokinase-Type Plasminogen Activator mRNA

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In LLC-PK$_1$ cells urokinase-type plasminogen activator (uPA) mRNA has a short half-life. It is stabilized by inhibition of protein synthesis and by downregulation of protein kinase C (PKC). In the present study on uPA mRNA metabolism, we focused our attention on the 3′ untranslated region (3′UTR) of the uPA mRNA, as this region is long and highly conserved among several mammalian species, including mice and humans. To investigate the possible role of the 3′UTR of uPA mRNA in mRNA metabolism, we inserted this region into the 3′UTR of the rabbit β-globin gene that is linked to the cytomegalovirus promoter and stably transfected it into LLC-PK$_1$ cells. While the parental globin mRNA was stable, the chimeric mRNA was degraded as rapidly as endogenous uPA mRNA, suggesting that the 3′UTR of uPA mRNA contains most of the information required for its rapid turnover. Further analysis showed that there are at least three independent determinants of instability in the 3′UTR; one is an AU-rich sequence located immediately 3′ of the poly(A) addition signal, and one is a sequence containing a stem structure. One determinant seems to require ongoing RNA synthesis for its activity. All chimeric unstable globin mRNAs became stable in the presence of cycloheximide, a protein synthesis inhibitor, suggesting that the stabilization of mRNA by protein synthesis inhibition is not through a specific sequence in the mRNA. In PKC-downregulated cells, globin mRNAs with the complete 3′UTR or the AU-rich sequence were stabilized, suggesting that PKC downregulation stabilizes uPA mRNA through the AU-rich sequence. Here we discuss the significance of multiple, independently acting instability determinants in the regulation of uPA mRNA metabolism.

The regulation of mRNA stability is thought to play an important role in eukaryotic gene expression through modulating the cytoplasmic abundance of mRNA. Different mRNAs have different half-lives in a given cell, and stability of the same mRNA can be temporally and spatially modulated (for reviews, see references 25, 37, 45, 52, 55, and 66). Little is known about the mechanism of mRNA degradation and its regulation. Several instability-determining sequences have been identified in the 3′ untranslated region (3′UTR) of many mRNAs: AU-rich sequences in various oncogene and lymphokine mRNAs (2, 13, 35, 58, 67), an iron-responsive element in transferrin receptor mRNA (14, 46), a cell cycle-dependent regulatory element in histone mRNA (41), and a Ca^{2+} sensitive regulatory element in granulocyte macrophage colony-stimulating factor (GM-CSF) mRNA (34). Instability-determining elements have also been identified in protein coding regions, e.g., c-*myc* (68), c-*fos* (59, 60), and *MATα1* (50) mRNAs. Proteins have also been identified which bind to the 3′UTR AU-rich element (9, 26, 42, 43, 49, 64, 70), to the iron-responsive element (36; for a review see reference 37), and to instability-determining regions in the protein coding regions of c-*myc* and c-*fos* mRNAs (8, 15). Some of these have been purified or characterized (26, 36, 42, 49), but their roles in the RNA degradation process have not been established. For functional analysis, cell-free mRNA decay reactions have been developed by several groups (3, 11, 51, 62, 65). By using functional assays, a cytosolic factor has been identified that binds to the c-*myc* AU-rich element and is copurified with specific c-*myc* mRNA-degrading activity (10). A regulatory role of the poly(A) tail in mRNA degradation has been suggested, because removal of the poly(A) tail precedes mRNA degradation (11, 59, 67). A poly(A)-degrading enzyme was detected in mammalian cells (6), and cDNA for PAN [poly(A)-binding protein-dependent poly(A) RNase] was cloned from yeast (56). However, the linkage of these activities or enzymes to specific mRNA degradation is not yet clear. So far, no RNase has been identified that specifically degrades mRNAs in eukaryotes.

We have been studying urokinase-type plasminogen activator (uPA) gene regulation in LLC-PK$_1$ cells, a cell line derived from porcine renal epithelia (33). In these cells, uPA mRNA is transcriptionally induced by the peptide hormone calcitonin or by 8-bromo-cyclic AMP (cAMP), both of which activate cAMP-dependent protein kinase, and by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), which activates protein kinase C (PKC) (18, 48). We have shown that uPA mRNA has a half-life of 70 min in LLC-PK$_1$ cells (4), and its stability is modulated not only by protein synthesis inhibition (3, 4) but also by PKC downregulation (72) and calcium ions (71), supporting the hypothesis that mRNA stability is as important a step as transcription in uPA expression. Indeed, mRNA stability is involved in the regulation of uPA expression in other cell types. Henderson et al. (28, 29) reported the posttranscriptional regulation of uPA gene expression in rat mammary tumor cells. We have shown that the stabilizing effect of cycloheximide is time dependent, although protein synthesis is immediately suppressed by the drug, advocating the presence of a labile protein factor involved in uPA mRNA degradation (3). Moreover, the cell-free mRNA decay system suggested that the labile factor is not free but is associated with mRNA (3).

To further analyze the mechanism of uPA mRNA degradation, we focused on the 3′UTR of the uPA mRNA, because this region is relatively long and highly conserved among five

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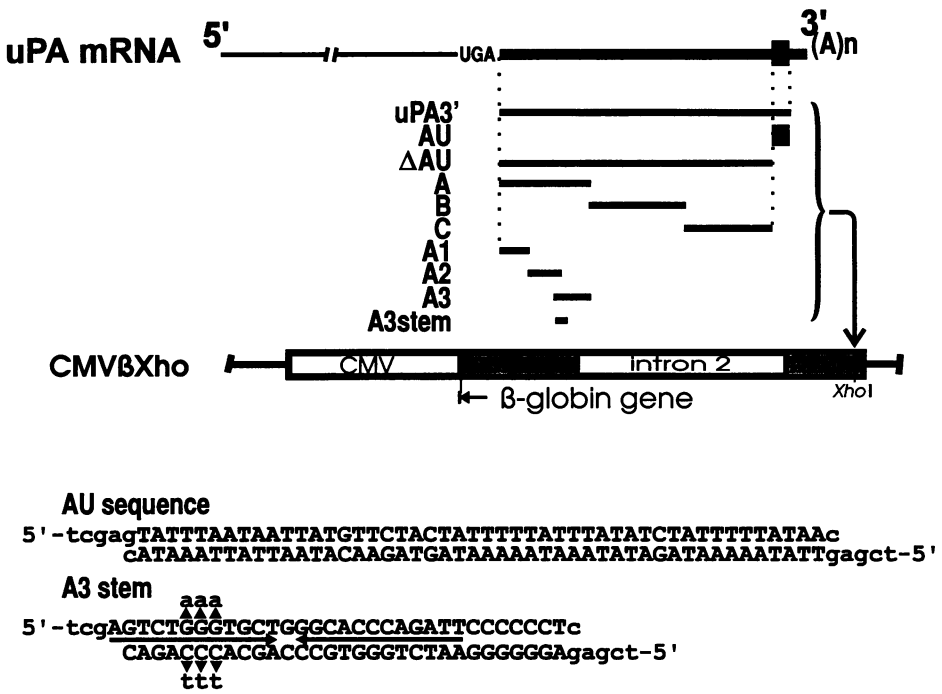

FIG. 1. Construction of chimeric β-globin genes. DNA fragments corresponding to parts of or the entire 3'UTR of pig uPA mRNA were prepared by PCR or chemical synthesis (for AU and A3stem) and were inserted into the *Xho*I site of the pCMVβXho expression vector. In the globin gene, coding regions and an intervening sequence are indicated. In the uPA mRNA, 3'UTR is indicated by a thick line; the AU-rich region is shown by a black box. Sequences of oligonucleotides for the AU-rich region and A3stem are shown at the bottom. The site of the potential stem structure is indicated by two arrows between the upper and lower sequences of A3stem in which G · U base pairing in RNA is taken into consideration. Mutation of A3stem (mutA3stem) is created by changing three nucleotides as indicated by arrowheads and lowercase letters above and below the A3stem sequences.

mammalian species (7, 27; also this work). The uPA 3'UTR was inserted into the 3'UTR of the globin gene linked to the cytomegalovirus (CMV) promoter vector and was stably transfected into LLC-PK_1 cells. We found that the uPA 3'UTR is sufficient to make otherwise stable globin mRNA highly unstable, to an extent comparable to that of the instability of endogenous uPA mRNA. We further characterized this region for instability and stabilization by protein synthesis inhibition and PKC downregulation.

MATERIALS AND METHODS

Materials. *Pfu* DNA polymerase was purchased from Stratagene, DNA polymerase I Klenow fragment was purchased from Biofinex, and DNA restriction enzymes were purchased from Biofinex and Boehringer Mannheim. Cycloheximide was purchased from Sigma, hygromycin was purchased from Calbiochem, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) was purchased from Fluka, and GeneScreen Plus was purchased from DuPont-New England Nuclear. [α-^{32}P]dATP (3,000 Ci/mmol) was obtained from Amersham.

Cell culture. LLC-PK_1 cells (33) were cultured in Dulbecco's modified Eagle's medium (Gibco-BRL) supplemented with 10% (vol/vol) fetal calf serum (AMIMED), 0.2 mg of streptomycin per ml, and 50 U of penicillin per ml at 37°C in a humidified CO_2 (6%) incubator.

Plasmids and probes. The plasmid pCMVβ was constructed by S. Wieland (Zürich, Switzerland); it contains the rabbit intron 1-less globin gene linked to the CMV promoter. pCMVβXho is the parent globin expression vector used in this work; it was derived from pCMVβ by mutating one base and thus creating a unique *Xho*I site 20 bp 5' of the poly(A) addition signal of the globin gene. Its derivatives were constructed by inserting PCR fragments of whole 3'UTR of uPA mRNA or parts of it into the *Xho*I site of pCMVβXho. Various globin genes were constructed by inserting different parts of the 3'UTR of uPA mRNA into the *Xho*I site of pCMVβXho. Oligonucleotides used to construct pCMVβ-AU and pCMVβ-A3stem are indicated in Fig. 1. Probes for Northern (RNA) blot hybridization were prepared by the standard random oligonucleotide-primed reaction (21) using purified cDNA inserts. The porcine cDNA clones for uPA, pYN15 (47), and actin, pAct4 (72), have been described. The rabbit β-globin cDNA clone pGEMglo was provided by E. Vakalopoulou (64).

Stable transfection. LLC-PK_1 cells were transfected by the calcium phosphate precipitation method (23) with recombinant globin genes together with pX343 (32), which carries a gene coding for hygromycin phosphotransferase. Stable transfectants were isolated by hygromycin selection, and more than 1,000 individual clones were pooled.

RNA isolation and Northern blot analysis. Total RNA (5 μg), isolated according to the method of Chomczynski and Sacchi (17), was resolved by agarose gel electrophoresis under denaturing conditions and was transferred to a nylon membrane as described previously (48). rRNA was stained on the filters with methylene blue (30) to assess RNA loading and transfer. Prehybridization and hybridization were performed as described previously (5), and filters were exposed to a Kodak X-Omat AR film with intensifying screens at −70°C.

Levels of specific RNA were quantitated by using a Molecular Dynamics PhosphorImager.

mRNA stability. RNA stability was measured by the RNA synthesis inhibitor-chase method as described previously (71). Briefly, cells were treated with DRB (20 μg/ml) to inhibit transcription, and total RNA was isolated at several time points thereafter. RNA was analyzed by Northern blot hybridization. Graphs were created by plotting values of mRNA levels with the SigmaPlot program (Jandel Scientific), and lines were drawn by using linear regression.

Nuclear transcription. The isolation of nuclei, nuclear run-on transcription, and quantitation of specific transcripts by hybridization were performed as described previously (5).

Southern blot analysis. Preparation of genomic DNA and its analysis by Southern blot hybridization were performed as described previously (48).

RESULTS

Presence of instability determinants in the 3′UTR of uPA mRNA. uPA mRNA is 2.4 kb long and contains 1 kb of 3′UTR. The sequence comparison of uPA mRNAs of five mammalian species, pigs (47), cows (39), humans (31, 54), rats (29), and mice (7), showed strikingly high homology not only in protein coding regions but also in 3′UTRs (data not shown). uPA 3′UTR has an AU-rich region immediately upstream of the poly(A) addition signal (29, 39, 47, 54). AU-rich sequences are found in many other short-lived mRNAs, such as oncogene and lymphokine mRNAs (13), and this sequence was shown to target these mRNAs for rapid degradation (2, 35, 58, 67). The high homology is not restricted to the AU-rich region but is observed throughout the sequence.

To find out whether these conserved sequences are important in the rapid turnover of uPA mRNA, we introduced into the 3′UTR of the rabbit globin gene different parts of the 3′UTR of pig uPA mRNA (Fig. 1). The three constructs tested encode globin-uPA3′, containing the entire uPA 3′UTR; globin-AU, containing only the AU-rich region of the uPA mRNA; and globin-ΔAU, containing the uPA 3′UTR without the AU-rich region. To ensure efficient expression in mammalian cells, the recombinant globin genes were linked to the CMV promoter. In each case, the promoters and globin translation units are identical, and therefore the transcripts derived from each construct differ only in their 3′UTRs. The stability of each hybrid globin mRNA was assessed by DRB-chase experiments. LLC-PK_1 cells stably transfected with these constructs were treated with DRB, which specifically inhibits the synthesis of eukaryotic heterogeneous nuclear RNA and mRNA (63), and total RNA was prepared at different times after the addition of the drug. The amount of globin mRNA was measured by Northern blot hybridization. The time course for degradation of the different globin mRNAs is shown in Fig. 2.

Globin-Xho mRNA did not decay over the 4-h time course of the experiment. In contrast, the hybrid globin mRNAs were unstable. The half-lives of these mRNAs were 8.5 h (globin-AU), 2.5 h (globin-ΔAU), and 1 h (globin-uPA3′). Thus, the presence of the uPA 3′UTR is sufficient to destabilize the otherwise stable globin message. From these data, we conclude that uPA 3′UTR contains most of the information determining instability and that not only the AU-rich region but also other regions contribute to the rapid turnover of uPA mRNA.

It should be noted that the basal level of each globin mRNA reflects its half-life: the more stable the transcripts the more abundant the mRNA, advocating the validity of the DRB-chase method. It may be argued that the basal mRNA levels reflect the copy numbers of integrated globin genes and that high basal levels of mRNA interfere with mRNA degradation. To exclude this possibility, we examined the copy number and transcription rate of integrated globin genes in each cell line. Southern blot analysis showed that the average copy numbers of integrated globin genes are almost the same for all cell populations except the one harboring pCMVβXho, which was slightly lower than the others (Fig. 2C). Likewise, nuclear run-on assays demonstrated that globin gene transcription rates were similar for all cell populations except the one harboring pCMVβXho, which was slightly lower than the others (Fig. 2D). Thus, these results confirm that basal levels of globin mRNAs reflect their half-lives.

Instability-determining sequences other than the AU-rich sequence. In order to localize the instability determinant residing upstream of the AU-rich region, ΔAU was further divided into three regions of about 280 bp each, designated A, B, and C (Fig. 1) and analyzed as above. In this experiment the half-life of globin-ΔAU was 1 h (Fig. 3), a somewhat lower value than that given in Fig. 2B. The basal level of globin-A mRNA was comparable to that of globin-ΔAU mRNA, but its half-life had doubled to 2 h (Fig. 3). The basal level of globin-B mRNA was quite low, but it did not show any decay in DRB-chase. The basal level of globin-C mRNA was high, and as expected it did not decay. As in previous experiments, the basal level of parental globin-Xho mRNA was high and did not display any decay (data not shown). These results suggest that region A contains an instability determinant but that this region alone is not enough to exhibit the instability-conferring effect to the degree observed with the entire ΔAU region.

The result with globin-B mRNA was surprising, because this RNA did not decay despite the fact that its basal level was quite low (Fig. 3). This was confirmed with independently transfected cell populations. This discrepancy could not be explained by low copy number. The copy number of integrated pCMVβ-B was slightly higher than that of the parental construct pCMVβXho (data not shown), and the transcription rate of CMVβ-B was also slightly higher than that of CMVβXho (data not shown). These data suggest that the B region has an instability determinant but that B-region-dependent degradation requires ongoing RNA synthesis.

A stem structure in region A. When region A was further analyzed by being divided into three regions, designated A1, A2, and A3 (Fig. 1), only the A3 region of 122 bases showed destabilizing activity (Fig. 4A). The half-life of globin-A3 was about 8 h, while that of globin-A was 2 h. The A3 region contains a sequence that is highly conserved among mammalian uPA mRNAs and has the potential to take a stem structure. When a 32-base part of the A3 region containing this sequence (A3stem [Fig. 1]) was tested by inserting two copies of it into pCMVβXho, the resulting globin-2×A3stem mRNA became unstable, with a half-life of 6 h (Fig. 4B). When the potential stem structure was disrupted by mutating three bases (Fig. 1), the resulting globin-2×mutA3stem mRNA became stable, and its steady-state level also increased.

Stabilization by protein synthesis inhibition. Previously, we had shown that inhibition of protein synthesis by cycloheximide or pactamycin stabilizes uPA mRNA in LLC-PK_1 cells (3, 4). To test whether this stabilizing effect is mediated by a particular region in the 3′UTR of uPA mRNA, cells harboring various globin gene constructs were treated with cycloheximide, and then the time course for mRNA accumulation was measured. As shown in Fig. 5, treatment with cycloheximide resulted in the accumulation of globin-uPA3′, globin-ΔAU, and globin-A and globin-B mRNAs. Globin-AU mRNA

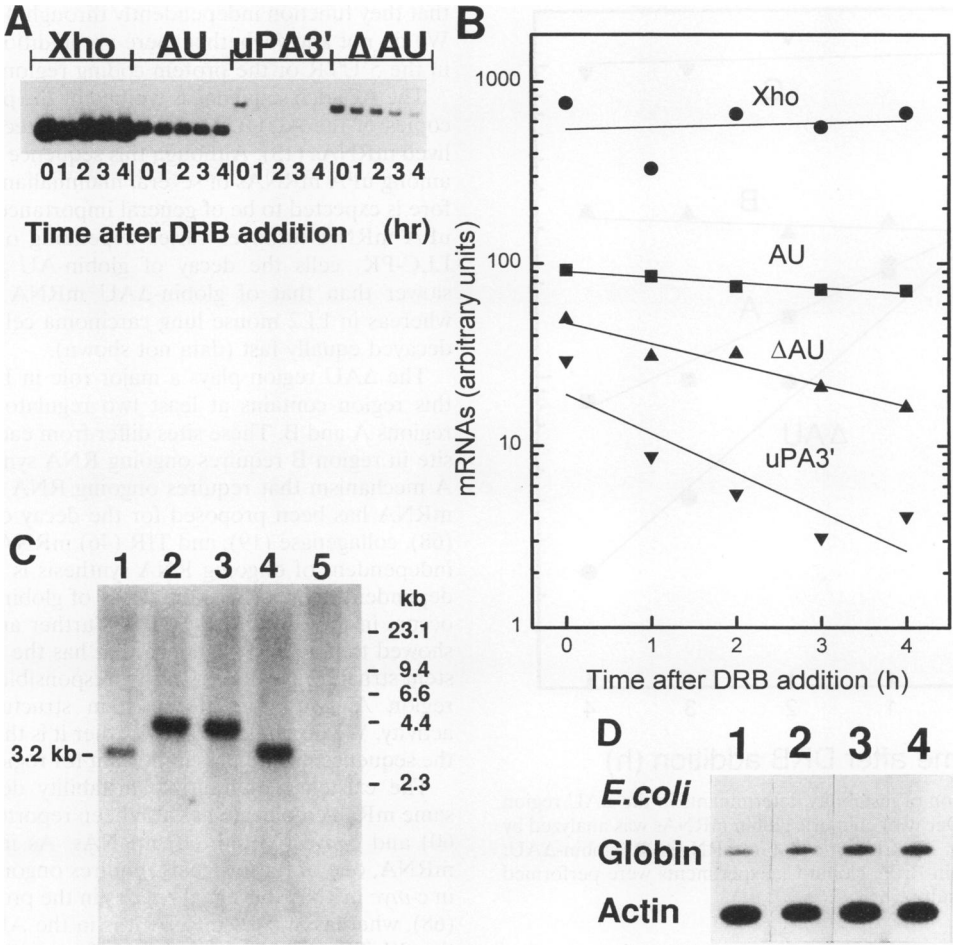

FIG. 2. Localization of RNA instability determinants in the uPA mRNA 3'UTR. (A) Cells stably transfected with various globin expression vectors were pooled and treated with DRB (20 μg/ml) for several periods indicated below the lanes. RNA was analyzed by Northern blot hybridization for globin mRNAs. Chimeric globin gene expression vectors are shown above each panel. Xho, AU, uPA3', and ΔAU denote pCMVβXho, -AU, -uPA3' and -ΔAU, respectively. Experiments were performed twice with similar results. (B) Northern blot filters were scanned by a PhosphorImager and values were plotted. Chimeric globin mRNAs: ●, globin-Xho; ■, globin-AU; ▲, globin-ΔAU; ▼, globin-uPA3'. (C) DNA was isolated from each transfectant and was analyzed by Southern blot hybridization using a globin cDNA fragment as a probe for the levels of chimeric gene insertion. Lanes 1 to 4, DNA (20 μg) from cells transfected with pCMVβXho, -uPA3', -ΔAU, and -AU, respectively; lane 5, DNA from nontransfected LLC-PK$_1$ cells. (D) Nuclear run-on transcription for transcription activities of chimeric globin genes. Nuclei were prepared from cells stably transfected with chimeric globin genes: lane 1, pCMVβXho; lane 2, pCMVβ-AU; lane 3, pCMVβ-uPA3'; lane 4, pCMVβ-ΔAU.

showed a delayed accumulation, and no obvious accumulation was detected with globin-C mRNA or parental globin-Xho mRNA (Fig. 5). The inductive effect of cycloheximide was not due to transcriptional activation, because the transcription rate of globin genes was not affected by cycloheximide, as shown by nuclear run-on assays (data not shown). The results suggest that the predominant effect of cycloheximide is on a posttranscriptional event and that the effect of cycloheximide is not through a specific sequence of mRNA.

uPA mRNA stabilization by PKC downregulation via the AU-rich sequence. Previously we showed that uPA mRNA is stabilized in LLC-PK$_1$ cells by PKC downregulation, which was induced by prolonged incubation of cells with higher concentrations of TPA (72). To examine which sequence element is responsible for this effect, we carried out DRB-chase experiments before and after PKC downregulation (Fig. 6). By PKC downregulation stationary levels of both globin-uPA3' and globin-AU mRNAs increased 2.5-fold. The half-life also increased from 30 min to 50 min for globin-uPA3' mRNA and from 10 h to >24 h for globin-AU mRNA. On the other hand, neither the stationary level nor the half-life of globin-ΔAU mRNA changed.

DISCUSSION

In the present work we have shown that the 3'UTR of uPA mRNA contains sufficient information for its rapid turnover. There are at least three regulatory sites within the 3'UTR, which appear to function independently: the AU-rich sequence and regions A and B (Fig. 3). The presence of a regulatory site in region B was inferred on the basis of the low steady-state level of globin-B mRNA, a lack of apparent difference in transcription, and the assumption that the DRB experiment was giving a false negative for rapid turnover because of a requirement for ongoing RNA synthesis (see below). Analysis of these sites with respect to their ability to mediate instability to a heterologous mRNA, sensitivity to protein synthesis inhibition, and sensitivity to PKC downregulation indicates

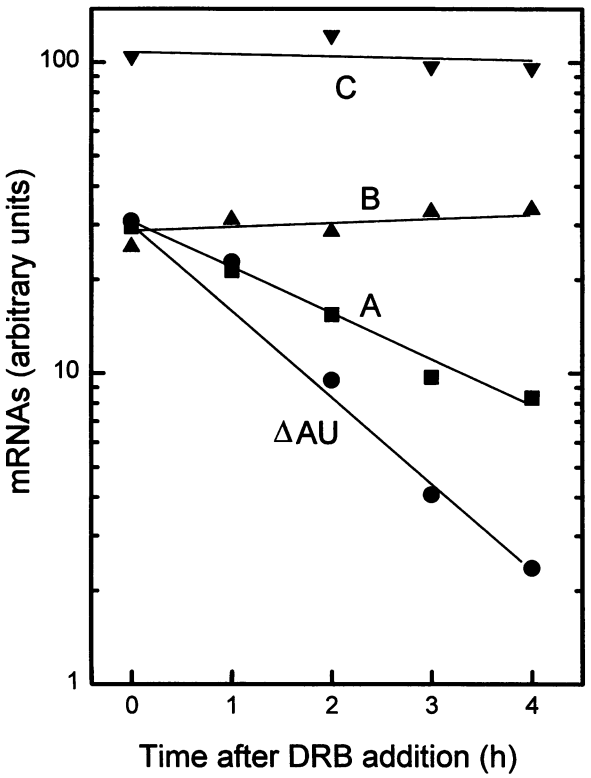

FIG. 3. Localization of instability determinants in the ΔAU region in the uPA 3′UTR. Decay of chimeric globin mRNAs was analyzed by DRB-chase as in Fig. 2B. Chimeric globin mRNAs: ●, globin-ΔAU; ■, globin-A; ▲, globin-B; ▼, globin-C. Experiments were performed twice with similar results.

that they function independently through distinct mechanisms. We do not know whether there are additional regulatory sites in the 5′UTR or the protein coding region.

The AU-rich sequence is typified by the presence of multiple copies of the AUUUA motif that is detected in many short-lived mRNAs (13). Although this sequence is highly conserved among uPA mRNAs of several mammalian species and therefore is expected to be of general importance, its significance in uPA mRNA turnover varies depending on the cell type. In LLC-PK$_1$ cells the decay of globin-AU mRNA was much slower than that of globin-ΔAU mRNA (Fig. 2A and B), whereas in LL2 mouse lung carcinoma cells the two mRNAs decayed equally fast (data not shown).

The ΔAU region plays a major role in LLC-PK$_1$ cells, and this region contains at least two regulatory sites present in regions A and B. These sites differ from each other in that the site in region B requires ongoing RNA synthesis to be active. A mechanism that requires ongoing RNA synthesis for rapid mRNA has been proposed for the decay of c-*fos* (60), c-*myc* (68), collagenase (19), and TfR (46) mRNAs. The mechanism independent of ongoing RNA synthesis is dominant over the dependent one, because the decay of globin-ΔAU mRNA still occurs in the presence of DRB. Further analysis of region A showed that a short sequence that has the potential to take a stem structure is, at least partly, responsible for the activity of region A. Mutation in the stem structure abrogated the activity. We do not know yet whether it is the stem structure or the sequence itself that is important for region A3stem activity.

The existence of multiple instability determinants in the same mRNA molecule has also been reported for c-*fos* (15, 59, 60) and c-*myc* (35, 40, 68) mRNAs. As in the case of uPA mRNA, one of these signals requires ongoing RNA synthesis; in c-*myc* mRNA the signal resides in the protein coding region (68), whereas in c-*fos* mRNA it is in the AU-rich sequence in the 3′UTR (60). Nevertheless, c-*fos* and c-*myc* mRNAs are

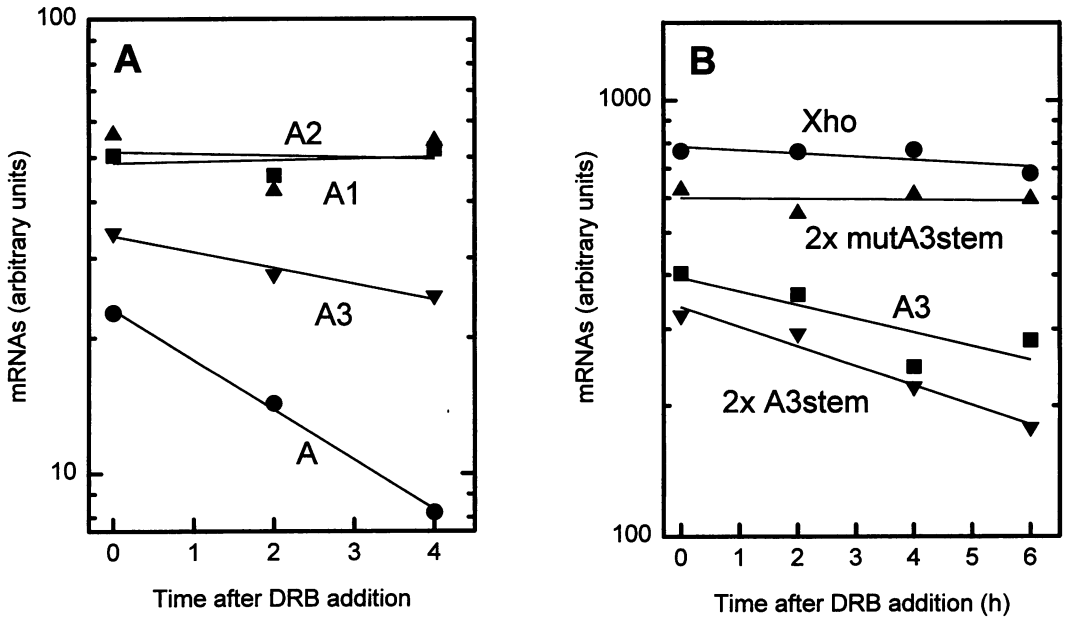

FIG. 4. Further analysis of region A. (A) Region A was further divided into three slightly overlapping regions, designated A1 (88 bases), A2 (104 bases), and A3 (122 bases) (Fig. 1) and was analyzed as in Fig. 2B. ●, globin-A; ■, globin-A1; ▲, globin-A2; ▼, globin-A3. (B) A short sequence in region A3 that has a conserved stem structure and its derivative (Fig. 1) were analyzed as in Fig. 2B. ●, globin-Xho; ■, globin-A3; ▼, globin-2×A3stem; ▲, globin-2×mutA3stem.

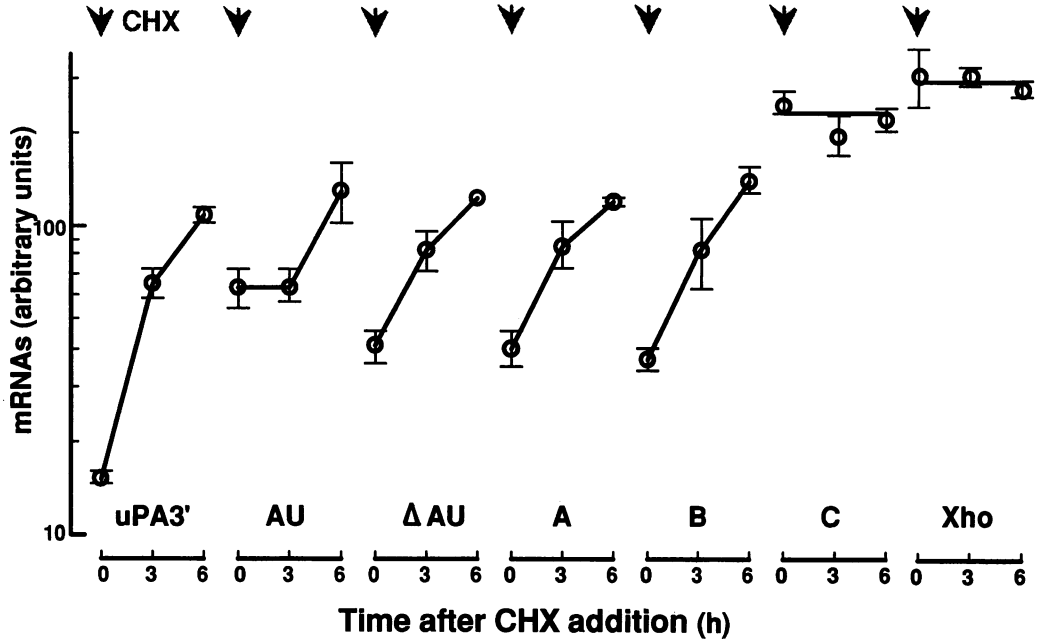

FIG. 5. Effect of cycloheximide on globin mRNA accumulation. Cells were treated with 10 μg of cycloheximide per ml for the times indicated. Total RNA was prepared and analyzed by Northern blot hybridization, and globin mRNAs were quantitated by a PhosphorImager. The experiment was done in duplicate, and the average values are shown with original values as error bars.

different from uPA mRNA, in which this sequence resides in region B, which does not contain the protein coding region or the AU-rich sequence. Whether these sites in three mRNAs share common biological and biochemical features is an interesting issue to be addressed in the future.

Many short-lived mRNAs are stabilized by the inhibition of protein synthesis. Two mechanisms, which are not mutually exclusive, are thought to account for the linkage of mRNA degradation with ongoing protein synthesis: (i) mRNA is degraded when it is undergoing translation, possibly because the mRNA-degrading machinery is tightly associated with the ribosome or because the translation product itself is involved in the degradation, and (ii) a labile protein factor is involved in mRNA degradation. There is evidence for both mechanisms. Studies using mutated mRNAs that contain a premature termination codon or a translation-inhibitory hairpin structure at the 5'UTR suggest translation-linked degradation of β-tubulin (69), cell cycle-dependent histone (24), c-*myc* (68), and GM-CSF (1, 57) mRNAs. In the case of β-tubulin mRNA, the signal is the first 4 codons (69), while in the case of c-*myc* mRNA one of the responsible sites is contained within the last 105 codons. Both mechanisms are proposed for c-*fos* mRNA stabilization by cycloheximide (20, 38, 67). uPA mRNA is stabilized by a protein synthesis inhibitor, cycloheximide, puromycin, or pactamycin, in LLC-PK_1 cells (3, 4). Previously we have presented data favoring the second possibility; the stabilizing effect of cycloheximide is time dependent, and in cell-free mRNA decay reactions, in which protein synthesis is severely impaired, uPA mRNA decays as fast as in vivo uPA mRNA, but it does not decay if cell extracts are prepared from cycloheximide-pretreated cells (3). Apparently, protein synthesis and mRNA degradation can be linked in various ways.

The levels of globin-AU mRNA increased after the addition of cycloheximide but with a delay, suggesting that a labile protein factor is involved in the mRNA degradation mediated by the AU-rich sequence (Fig. 5). On the other hand, the levels

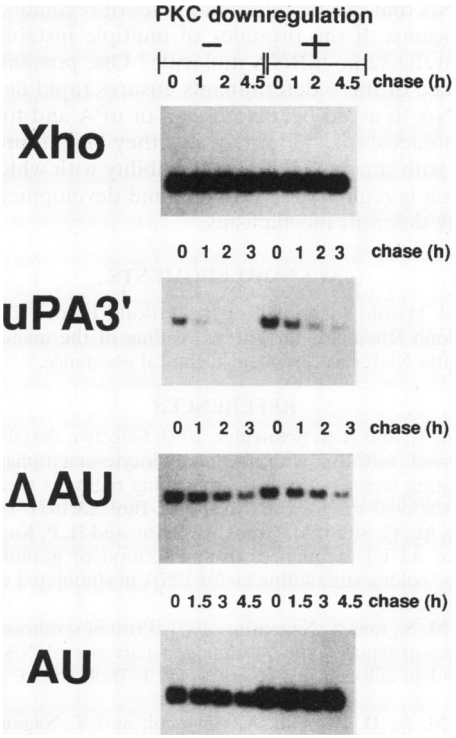

FIG. 6. Effect of PKC downregulation on globin mRNA stability. Cells were pretreated for 24 h with 0.05% dimethyl sulfoxide, a vehicle of TPA (−), or with 100 ng of TPA per ml (+). Next, DRB was added and at the times indicated above the lane total RNA was prepared. RNA was analyzed by Northern blot hybridization for globin mRNAs. Xho, uPA3', ΔAU, and AU denote globin-Xho, -uPA3', -ΔAU and -AU mRNAs, respectively.

of other chimeric globin mRNAs, including globin-3′UTR mRNA, increased without delay after the addition of cycloheximide, suggesting that the cessation of translation itself is responsible for the stabilization of these mRNAs and that the time-dependent effect actuated via the AU-rich sequence is overrun by the regulation mediated by regions other than the AU-rich sequence.

We have previously shown that PKC downregulation increases the half-life of cAMP-induced uPA mRNA (72). Likewise, both globin-3′UTR and globin-AU mRNAs, but not globin-ΔAU mRNA, became stable in PKC-downregulated cells, suggesting that the AU-rich sequence is responsible for PKC downregulation-induced uPA mRNA stabilization and that cAMP does not have an active role in this process. Akashi et al. (2) reported that stabilization of GM-CSF mRNA by PKC activation is mediated by AU-rich sequences. Recently, Chen et al. (16) identified a region in the 3′UTR of ribonucleotide reductase R1 mRNA and a protein of 52 to 57 kDa interacting with this region that are involved in TPA-induced stabilization. Stabilization after TPA treatment has also been reported for collagenase (12), interleukin 1 (22), GAP-43 (53), GLUT1 (61), and TGFβ1 (65) mRNAs. uPA mRNA is unique in that it is PKC downregulation that causes stabilization of uPA mRNA through the AU-rich sequence. Malter et al. (44, 61) have shown that TPA induces adenosine-uridine binding factor (AUBF) activity in the cytoplasm and have suggested that AUBF has a protective effect. Several proteins binding to AU-rich sequence have been identified (26, 42, 49). It would be interesting to see if one of these proteins changes its behavior after PKC downregulation.

We have identified three instability-determining sites in the uPA mRNA that exhibit different modes of regulation. What is the significance of the presence of multiple instability determinants in the same mRNA molecule? One possibility is that the presence of these determinants ensures rapid depletion of uPA mRNA to avoid overexpression of uPA and to maintain cellular homeostasis. Alternatively, they might provide the organism with the versatility and flexibility with which mRNA degradation is regulated in a tissue- and development-specific manner by different mechanisms.

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