cAMP-dependent stabilization of phosphoenolpyruvate carboxykinase mRNA in LLC-PK₁-F⁺ kidney cells

Purabi S. Dhakras, Sachin Hajarnis, Lynn Taylor, and Norman P. Curthoys
Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, Colorado
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Dhakras, Purabi S., Sachin Hajarnis, Lynn Taylor, and Norman P. Curthoys. cAMP-dependent stabilization of phosphoenolpyruvate carboxykinase mRNA in LLC-PK₁-F⁺ kidney cells. Am J Physiol Renal Physiol 290: F313–F318, 2006. First published September 6, 2005; doi:10.1152/ajprenal.00249.2005.—Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes a rate-limiting step in hepatic and renal gluconeogenesis. In the kidney, PEPCK expression is enhanced during metabolic acidosis and in response to ANG II and parathyroid hormone. The effect of the latter hormone is mediated, in part, by cAMP. Treatment of subconfluent cultures of LLC-PK₁-F⁺ cells, a gluconeogenic line of porcine proximal tubule-like cells, with cAMP produces a pronounced increase in the level of PEPCK mRNA. The luciferase activity of pLuc/3'-UTR-1, a reporter construct that contains the 3'-UTR of the PEPCK mRNA, was increased three- to fourfold by coexpression of the catalytic subunit of protein kinase A (PKA). This result indicates that cAMP-dependent stabilization may contribute to the increased expression of PEPCK mRNA in LLC-PK₁-F⁺ cells. Various pLuc/3'-UTR constructs containing different segments of the 3'-UTR of PEPCK mRNA were used to map the cAMP response to two segments that were previously shown to bind AUFI and to function as instability elements. A tetracycline-responsive promoter system was used to quantify the effect of forskolin on the half-lives of chimeric β-globin-PEPCK (TβG-PCK) mRNAs. The half-life of the labile βG-PCK-1 mRNA was increased eightfold by addition of forskolin. In contrast, the half-lives of the constructs containing the individual instability elements were increased only twofold. Therefore, the multiple instability elements present within the 3'-UTR may function synergistically to mediate both the rapid degradation and the cAMP-induced stabilization of PEPCK mRNA. The latter process may result from a PKA-dependent phosphorylation of AUFI.

proximal tubule; messenger ribonucleic acid stability; AUFI; forskolin; adenosine 3',5'-cyclic monophosphate

THE CYTOSOLIC ISOFORM of phosphoenolpyruvate carboxykinase (PEPCK) is expressed in a tissue- and cell-specific manner that is developmentally regulated (10). The enzyme is expressed predominantly in liver, kidney, and adipose tissue where it functions as a key regulator of gluconeogenesis, ammoniagenesis, and glyceroneogenesis, respectively. However, this activity is not regulated by allosteric mechanisms or covalent modifications (10). Instead, the level of the PEPCK protein is determined by mechanisms that regulate the rate of synthesis and degradation of the PEPCK mRNA. Transcription of PEPCK mRNA is controlled by a variety of hormones including insulin, thyroid hormone, glucocorticoids, and glucagon (9). The latter hormone acts through cAMP-dependent activation of protein kinase A (PKA). Within the renal proximal tubule, parathyroid hormone increases cAMP levels and causes an increased expression of PEPCK (19).

Various experiments indicate that cAMP also inhibits degradation of the PEPCK mRNA. Administration of cAMP, only transiently, enhanced transcription of the PEPCK gene in rat liver (15) and in H4IIE hepatoma cells (20) while the amount of PEPCK mRNA continued to accumulate in the cytoplasm. Further studies in FTO-2B rat hepatoma cells demonstrated that the immediate effect of cAMP is to cause an increase in transcription, whereas the long-term effect occurs mainly through stabilization of the PEPCK mRNA (11). The rate of transcription was increased fourfold within 5 min after addition of Bt2cAMP, remained constant for 20 min, and then declined. However, the level of PEPCK mRNA increased gradually, was induced sixfold after 90 min, and then remained at this level. Therefore, a change in the abundance of PEPCK mRNA did not correlate with the changes in the rate of transcription. Subsequent pulse-chase analysis indicated that treatment with cAMP caused a sixfold stabilization of the PEPCK mRNA in FTO-2B cells (11).

LLC-PK₁-F⁺ cells are a gluconeogenic line of porcine renal proximal tubule-like cells (7) that express significant levels of PEPCK (12). The cells effectively model both the cAMP-dependent (17) and the pH-responsive (14) changes in cytosolic PEPCK gene expression that occur in rat kidney. Treatment of LLC-PK₁-F⁺ cells with CPT-cAMP causes a rapid and pronounced (21-fold) induction of the PEPCK mRNA that is due, at least in part, to activation of transcription (17).

The 3'-untranslated region (UTR) of the PEPCK gene contains 615 bp that include both AU-rich and CU-rich regions that play an important role in the rapid turnover of the PEPCK mRNA (16). A tetracycline-responsive promoter system was used to quantify the turnover of various chimeric β-globin-PEPCK (TβG-PCK) mRNAs in LLC-PK₁-F⁺ cells. The control TβG mRNA was extremely stable (half-life = 5 days) while TβG-PCK-1 mRNA that includes the 3'-UTR of the PEPCK mRNA was degraded with a half-life of 2 h (8). Further analysis established that the 5'-half (PCK-2 segment) and the 73-nt 3'-end (PCK-6/7 segment) contained instability elements that bind AUFI and that the two elements act synergistically to produce the rapid degradation characteristic of PEPCK mRNA.

In the current study, a cAMP-dependent activation of a luciferase reporter construct containing the PCK-1 segment was observed and this effect was mapped to the PCK-2 and PCK-6/7 segments. In addition, the tetracycline-responsive promoter system was used to demonstrate that the half-life of the TβG-PCK mRNA increased eightfold by addition of...
forskolin, whereas the corresponding PCK-2 and PCK-6/7 constructs were stabilized only twofold. Therefore, the cAMP-dependent stabilization of the PEPCK mRNA is mediated through the synergistic interaction of the two instability elements and may involve phosphorylation of AUFI.

MATERIALS AND METHODS

Materials. [α-32P]dCTP (3,000 Ci/mmol) was obtained from MP Biologicals. The Dual Luciferase Reporter Assay Kit was obtained from Promega. Forskolin and doxycycline (Dox) were obtained from Sigma. Hygromycin B was purchased from Mediatech. The GENECLEAN Kit was purchased from Bio101, and the oligolabeling kit was from Ambion. GeneScreen Plus was purchased from Perkin Elmer Life Sciences. The expression vector for the catalytic subunit of PKA was obtained from W. J. Roessler (University of Saskatchewan). Other chemicals were acquired from Fisher, New England Biolabs, or Sigma.

Construction of luciferase vectors. The pLuc/Zeo plasmid was designed to facilitate the rapid detection and mapping of mRNA instability elements (16). It contains the cytomegalovirus (CMV) promoter, the entire coding region of the firefly luciferase gene, a segment of the 3'-UTR of the Simian Virus 40 (SV40) containing a single intron, a multicloning site containing nine unique restriction sites, and the 3'-UTR and polyadenylation site of the bovine growth hormone gene. The pLuc-PCK-1/Zeo plasmid also contained a 593-bp segment of the 3'-UTR of the PEPCK gene. The only portion of the 3'-UTR omitted from the PCK-1 segment was the terminal AAAAAAAAACCTTTTATAGAAA sequence that included the polyadenylation site (underlined). However, the segment of SV40 cDNA may encode an instability element and hence this sequence was removed to produce an improved vector. The initial plasmid pLuc/Zeo was digested with HindIII to produce a 5-kb fragment and a 1.7-kb segment containing the luciferase coding region and the SV40 3'-UTR. Primers were designed to amplify the luciferase coding region. The 1,649-bp PCR product, containing the luciferase coding region without the SV40 sequence, was subcloned into pPCR-Script, which contained the 1,7-kb segment containing the luciferase coding region and the SV40 3'-end without the SV40 sequence, was subcloned into pPCR-Script, which contains the 1,7-kb segment containing the luciferase coding region and the SV40 3'-UTR. 

The resultting plasmid was named pLuc/3'-PCK-1, which contains the PCK-1 segment of the PEPCK mRNA, was synthesized in a similar manner. The original plasmid pLuc-PCK-1/Zeo was digested with HindIII to produce a 5.6-kb vector fragment. The 1,649-bp PCR product containing the luciferase coding region was ligated into the vector fragment to produce pLuc/3'-PCK-1. The remaining constructs, pLuc/3'-PCK-2, pLuc/3'-PCK-3, pLuc/3'-PCK-6, pLuc/3'-PCK-7, and pLuc/3'-PCK-6/7, were synthesized from the corresponded pLuc/Zeo plasmids (16) using the same protocol.

Luciferase assay. The 507-bp fragment of rabbit β-globin cDNA was excised by restriction with BglII and HindIII from plasmid pAr2 (4) and inserted into HindIII and BglII sites of pTRE2 (Clontech). This construct contains a tetracycline-responsive promoter that contains the PEPCK mRNA in LLC-PK1-F314 cells (7). The constructs were transformed into E. coli JM109, and the resulting plasmids were purified and sequenced. The resulting plasmids were designated pLuc3-PCK constructs.

Construction of TetβG-PCK vectors. A tetracycline-regulated promoter system (24) was used to quantify the effect of forskolin on the half-lives of various chimeric PEPCK mRNAs. A chimeric β-globin/growth hormone gene was cloned into the pTRE2 plasmid (Clontech) to yield pTetβG (8). This construct contains a tetracycline-responsive promoter, the coding region of the rabbit β-globin (βG) gene containing three exons and two introns, a multicloning site, and the bovine growth hormone (bGH) 3'-UTR and polyadenylation site. The pTβG-PCK-1 construct was produced by inserting the 593-bp 3'-UTR segment of PEPCK cDNA into the multicloning site of pTetβG (8). Similarly, pTβG-PCK-2 contains a 369-bp segment from the 5'-end of PCK-1 while pTβG-PCK-6/7 contains the 74-bp segments that constitutes the 3'-end of PCK-1.

Half-life analysis. To characterize the segment within the 3'-UTR of the PEPCK mRNA that mediates the cAMP-dependent stabilization, half-life analyses were performed using the 5'UTR of LLC-PK1-F314 cells (7). A terminal tTA and strongly inhibits transcription. With the use of this system, transcription of a single gene can be fully induced and then rapidly repressed to create a pulse of newly synthesized mRNA. Once transcribed, decay of the reporter mRNA was followed by Northern blot analysis of RNA isolated at different times after addition of Dox.

Northern analysis. Subclones of the 8C cells that stably express the various pTβG-PCK plasmids (8) were grown in medium containing 50 ng/ml of Dox. Twelve hours before the creation of a transcriptional pulse, 0.1 mM forskolin was added to the experimental plates and an equivalent amount of DMSO was added to the control plates. Medium without Dox, but containing forskolin or DMSO, was subsequently added. After 3 h, the 0-h RNA samples were isolated using TRIzol reagent (Invitrogen) and fresh medium containing 1 µg/ml of Dox was added along with forskolin or DMSO to the remaining plates to inhibit transcription. RNA samples were isolated at 4-h intervals and were quantified by Northern blot analysis. For the pulse-chase experiments, cells were grown for 48 h in the absence of Dox and RNA was isolated at various times after addition of 1 µg/ml of Dox. A 507-bp fragment of rabbit β-globin cDNA was excised by restriction with MvaI and HindIII. The fragments were separated on agarose gels, excised, and purified using the GENECLEAN kit. The synthesis of oligolabeled cDNA probes and Northern blot analysis were performed as described previously (16). The blots were exposed to a PhosphorImager screen and quantified using Molecular Dynamics Software. The level of the chimeric β-globin mRNA was divided by the level of the corresponding βG mRNA to correct for errors in sample loading. The log of normalized data was then plotted vs. the time after addition of Dox. The values are means ± SE of data obtained from triplicate samples. The line represents the best-fit of the data points as determined by the Kaleidograph program that weighs each data point based on its standard deviation.

RESULTS

Mapping of the 3'-instability and cAMP response elements. The various pLuc3'-PK constructs were used to assess whether an increase in endogenous cAMP affects the stability of the PEPCK mRNA in LLC-PK1-F314 cells. When transfected in equivalent amounts, pLuc3'-PCK-1 produced only 15% of the luciferase activities obtained from the various pLuc3'-PCK constructs were used to measure the activity of the Renilla luciferase activities due to changes in transcription efficiency. In a single experiment, each transfection was performed in triplicate. For comparative purposes, the mean of the ratio of luciferase activities measured with the parent construct was normalized to a value of one. This arbitrary unit was selected so that a normalized value of the experimental construct of less than one indicates a decrease in the stability of the chimeric mRNA.

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the luciferase activity exhibited by pLuc3′ (Fig. 1, open bars). This result is consistent with the presence of multiple instability elements within the PCK-1 segment (8). Cotransfection of pLuc3′ with a plasmid that encodes the catalytic subunit of PKA had no significant effect on the luciferase activity. By contrast, the luciferase activity of the pLuc3′-PCK-1 construct was increased three- to fourfold (Fig. 1, filled bars). This observation suggests that the large increase in PEPCK mRNA produced by treatment of LLC-PK1-F + cells with cAMP may be due, in part, to stabilization of the PEPCK mRNA.

The relative luciferase activities of the various pLuc3′ constructs were subsequently analyzed. The schematic diagram (Fig. 2A) illustrates the segments of the 3′-UTR of the PEPCK mRNA that are encoded in the various pLuc3′ constructs and the known locations of instability elements that bind AUF1. In this experiment, pLuc3′-PCK-1 exhibited only 12% of the activity produced by pLuc3′ (Fig. 2B, open bars). In contrast, pLuc3′-PCK-2, which contains the 5′-half of the 3′-UTR, and pLuc3′-PCK-3, which contains the 3′-half, exhibit ~40% of the activity of pLuc3′. This observation is consistent with the previous conclusion that both segments contain instability elements that function synergistically to produce the rapid turnover of the PEPCK mRNA (8). AUF1 was shown to bind to separate sites within the PCK-3 segment that were termed PCK-6 and PCK-7. However, neither pLuc3′-PCK-6 nor pLuc3′-PCK-7 exhibits a decrease in luciferase activity when compared with pLuc3′. However, pLuc3′-PCK-6/7 produced the same decrease in luciferase activity as observed with pLuc3′-PCK-3.

The effect of coexpression of the catalytic subunit of PKA on the luciferase activities of various pLuc3′-PCK constructs was also determined (Fig. 2B, filled bars). Again, pLuc3′-PCK-1 exhibited a threefold increase in luciferase activity in the presence of PKA. However, the pLuc3′-PCK-2 and pLuc3′-PCK-3 exhibited only a twofold increase in luciferase activity. In contrast, pLuc3′-PCK-6 and pLuc3′-PCK-7 did not show a significant increase in the luciferase activity in the presence of PKA. However, a twofold response was observed with pLuc3′-PCK-6/7. These results suggest that both the 5′- and 3′-instability elements contribute to the cAMP-responsive stabilization of the PEPCK mRNA. pLuc3′-GA, a reporter construct containing the 3′-UTR of the glutaminase (GA) mRNA, was used as a control. The level of GA mRNA is not induced by treatment of LLC-PK1-F + cells with cAMP (13). Similarly, the luciferase activity of pLuc3′-GA was not affected by coexpression of PKA.

Half-life analysis. For half-life analysis, forskolin, an activator of adenylate cyclase, was used to increase the endogenous steady-state levels of cAMP. Increasing concentrations of forskolin produced increases in the luciferase activity of pLuc3′-PCK-1 that are similar to those observed previously by coexpression of PKA (data not shown). However, forskolin concentration above 0.3 mM caused cell death. Therefore, cells were treated with 0.1 mM of forskolin. A tetracycline-regulated promoter (Tet-off) system was used to characterize the ability of the various segments to function as instability elements and to respond to cAMP.

The half-life of the TβG-PCK-1 mRNA was determined using a pulse-chase protocol. Cells were grown in the presence of 50 ng/ml Dox to maximally suppress expression of the transgene. A pulse of newly synthesized TβG-PCK-1 mRNA was created by removing the Dox for 3 h. Following addition of 1 μg/ml Dox, the subsequent decay of the TβG-PCK-1 mRNA was determined by Northern blot analysis. With the use of this protocol, it was determined that the TβG-PCK-1 mRNA decayed with a half-life of 2 h. However, in the presence of 0.1 mM forskolin, the TβG-PCK-1 mRNA was greatly stabilized and decayed with a half-life of 16 h (Fig. 3).

To utilize the pulse-chase protocol generally requires identification of a cell line that exhibits at least a 50-fold difference in mRNA expression when grown in the presence or absence of Dox. The cell lines that stably express the TβG-PCK-2 and TβG-PCK-6/7 mRNAs exhibited only a 5- to 10-fold induction on removal of Dox. Therefore, the half-life analyses of these mRNAs were performed without a transcriptional pulse. Cells were grown in the absence of Dox to obtain maximal expression of the transgene, and RNA samples were isolated at 0, 4, 8, and 12 h after addition of 1 μg/ml of Dox. Northern blot analysis indicated that the TβG-PCK-2 mRNA was degraded with a half-life of around 5 h. However, in the presence of 0.1 mM forskolin, the half-life was increased to 9 h (Fig. 4). Similarly, the TβG-PCK-6/7 mRNA was degraded with a half-life of around 4.5 h, while in the presence of 0.1 mM forskolin the half-life was 9.8 h (Fig. 5). Therefore, the latter constructs exhibit a slight but significant cAMP-dependent stabilization. However, neither construct reproduces the large response that is observed with the full-length TβG-PCK-1.
DISCUSSION

In the present study, an improved luciferase reporter construct was used to map the segments of the 3'-UTR that participate in the cAMP-dependent stabilization of the PEPCK mRNA. Insertion of the PCK-1 segment into the pLuc/Zeo reporter plasmid resulted in only a 2.5-fold decrease in luciferase activity when expressed in LLC-PK1-F cells (16). By contrast, the pLuc/3'-PCK-1 construct exhibited a sevenfold decrease in luciferase activity compared with the pLuc/3'-PCK-2 construct. Consistent with the recent mapping of multiple instability elements within the PCK-1 segment (8), both pLuc/3'-PCK-2 and pLuc/3'-PCK-3 exhibited a reduced but significant decrease in luciferase activity. As anticipated, neither the PCK-6 nor the PCK-7 segment was sufficient to produce a decrease in luciferase activity compared with pLuc/3'. However, the combined segments (PCK-6/7) produced a decrease in luciferase activity that was equal to that observed with the pLuc/3'-PCK-3 construct. This result is consistent with the previous conclusion that both the PCK-6 and PCK-7 binding sites are required to fully reconstitute the 3'-instability element of the PEPCK mRNA (8).

Coexpression of the catalytic subunit of PKA resulted in a significant three- to fourfold increase in the luciferase activity of pLuc/3'-PCK-1 but had no effect on the pLuc/3' activity (Fig. 1). The sole difference between the two plasmids is the addition of the PCK-1 sequence within the 3'-UTR of the pLuc/3' gene. Therefore, a cAMP-dependent stimulation of transcription or an effect on the activity or stability of the luciferase protein should cause a similar response in the activities of both constructs, whereas an effect that is specific to the pLuc/3'-PCK-1 construct is indicative of altered mRNA stability. The luciferase activities of pLuc/3'-PCK-2 and pLuc/3'-PCK-6/7 were enhanced almost twofold in the presence of PKA. Thus the two segments that bind AUFI and determine the instability may also mediate the cAMP-responsive stabilization of the PEPCK mRNA. Thus the observed stimulation of the various pLuc/3'-PCK constructs by cAMP provides a rapid method to screen for potential elements that affect mRNA stabilization.
stability. However, definitive conclusions regarding hormonal effects on mRNA stability require the measurement the half-life of the endogenous mRNA or a suitable reporter construct.

In previous studies conducted with hepatoma cells, the effect of cAMP on the half-life of the PEPCK mRNA was characterized by pulse-chase analysis using a [32P]labeled nucleotide (11). The addition of cAMP caused a sixfold increase in the half-life of the PEPCK mRNA. In the current study, a tetracycline-regulated promoter system was used to study the effect of cAMP on the half-life of PEPCK mRNA in LLC-PK1-F

The cell lines that stably express the TβG-PCK-2 and TβG-PCK-6/7 mRNAs exhibit only a 5- to 10-fold increase in either mRNA on removal of Dox. As a result, the half-lives of the TβG-PCK-2 and TβG-PCK-6/7 mRNAs were analyzed without a transcriptional pulse. As a control, the TβG-PCK-1-expressing cells were subjected to half-life analysis with and without the formation of a transcriptional pulse. The two

Fig. 3. Effect of forskolin on the half-life of chimeric β-globin-PEPCK (TβG-PCK-1) mRNA. Cells were treated with or without 0.1 mM forskolin. To create a transcriptional pulse, cells were initially grown in medium containing 50 ng/ml of doxycycline (Dox) and then transferred to medium minus Dox for 3 h. Transcription of the TβG-PCK-1 gene was subsequently inhibited by addition of 1 μg/ml of Dox and cells were harvested at various intervals. The levels of the chimeric mRNAs and the 18S rRNAs were quantified by Northern analysis (top). The levels of the TβG-PCK-1 mRNAs were divided by the corresponding levels of 18S rRNAs to correct for errors in sample loading. The log of the normalized data was plotted against the time after Dox addition (bottom). The reported data are means ± SE of triplicate samples from a single experiment and are representative of the results obtained from 2 experiments.

Fig. 4. Half-life analysis of TβG-PCK-2 mRNA in the presence and absence of 0.1 mM forskolin. Cells were grown for 48 h in the absence of Dox, after which 1 μg/ml of Dox was added to shut off transcription. RNA was isolated at various times after addition of Dox and the levels of the chimeric βG mRNAs and 18S rRNAs were quantified by Northern analysis (top). The log of normalized data was then plotted against the time after Dox addition (bottom). The reported data are means ± SE of triplicate samples from a single experiment and are representative of the results obtained from 2 experiments.

Fig. 5. Half-life analysis of TβG-PCK-6/7 mRNA in the presence and absence of 0.1 mM forskolin. Cells were grown for 48 h in the absence of Dox, after which 1 μg/ml of Dox was added to shut off transcription. RNA was isolated at various times after addition of Dox and the levels of the chimeric βG mRNAs and 18S rRNAs were quantified by Northern analysis (top). The log of normalized data was then plotted against the time after Dox addition (bottom). The reported data are means ± SE of triplicate samples from a single experiment and are representative of the results obtained from 2 experiments.
protocols produced identical results (data not shown) indicating that the measured half-life was independent of the method of analysis. The TβG-PCK-2 and TβG-PCK-6/7 mRNAs were degraded with half-lives of 4–5 h and were stabilized nearly twofold by addition of forskolin. Thus the two segments cause a significant destabilization of the TβG mRNA and exhibit a slight, but significant, cAMP-dependent stabilization. However, neither the TβG-PCK-2 mRNA nor the TβG-PCK-6/7 mRNA exhibits the rapid turnover and the eightfold cAMP-dependent effect that are observed with the TβG-PCK-1 mRNA. A more detailed mapping analysis indicated that conserved sequences within the PCK-2 and PCK-6/7 segments mediate the rapid turnover of the PEPCk mRNA in kidney cells (8). The results of the current study suggest that the same sequences may be involved in the cAMP-dependent stabilization of the PEPCk mRNA. Therefore, the multiple instability elements present within the 3’-UTR may function synergistically to mediate both the rapid degradation and the cAMP-induced stabilization of PEPCk mRNA.

Recent experiments identified AUF1 as one of the proteins that bind to the specific elements within the 3’-UTR that mediate the rapid degradation of PEPCk mRNA in LLC-PK1-F cells (8). Four isoforms (45, 42, 40, and 37 kDa) of AUFI are produced by alternative splicing of its initial transcript (21). Each isoform contains a dimerization domain in its N-terminal region, two RNA recognition motifs (RRM), which make contacts with the appropriate RNA substrates, and a glutamine-rich domain (Q-rich domain), which is important for sequence-specific oligomerization of AUFI when bound to RNA substrates. The two larger isoforms contain a binding determinant for components of the nuclear scaffold and are largely retained in the nucleus (1). In contrast, the p37 and p40 isoforms of AUFI lack this determinant and shuttle between the nucleus and cytoplasm (5). AUFI has been shown to bind to various AU-rich elements (AREs) (3) and may recruit the exosome, a complex consisting of ribonucleases, that accounts for the rapid degradation of specific mRNAs in eukaryotic cells (18). Recent data suggest that selective phosphorylation of AUFI may regulate ARE-directed mRNA turnover by remodeling local RNA structures, potentially altering the presentation of RNA and/or protein determinants involved in subsequent trans-factor recruitment (23).

PKA has been shown to phosphorylate AUFI and regulate ARE-directed mRNA turnover (22). It was observed that the recombinant p40 isoform of AUFI was quantitatively phosphorylated in vitro by PKA at a specific serine residue. Furthermore, posttranslational modification of AUFI was observed to regulate its ARE-targeting role and alter the AUFI-ARE complex conformation and dynamics. These observations provide a potential mechanism for inhibition of ARE-directed mRNA turnover. The association of both unphosphorylated and phosphorylated AUFI with polysomes also suggested that this modification might serve as a switching mechanism, which converts AUFI from an “mRNA-destabilizing” to an “mRNA-stabilizing” factor. Thus cAMP-dependent activation of PKA in kidney cells may result in phosphorylation of the AUFI protein, which in turn affects its targeting to AREs or its ability to interact with other proteins that lead to the stabilization of PEPCk mRNA.

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