# Cytosolic phospholipase $A_2$ gene expression in rat mesangial cells is regulated post-transcriptionally

Agnes TAY, Peter MAXWELL, Zhen-Guo LI, Howard GOLDBERG and Karl SKORECKI*

Membrane Biology Group, Toronto Hospital and Hospital for Sick Children Research Institutes, Division of Nephrology, University of Toronto, Toronto, Canada M5G 2C4

Cytosolic phospholipase $A_2$ ($cPLA_2$) is thought to be the rate-limiting enzyme in the arachidonic acid/eicosanoid cascade. The ability of various agonists to increase steady-state $cPLA_2$ mRNA levels has previously been reported. The current study delineates the contributions of transcriptional and post-transcriptional processes to the regulation of $cPLA_2$ gene expression in response to a variety of agonists in cultured rat glomerular mesangial cells. Epidermal growth factor, platelet-derived growth factor, serum and phorbol myristate acetate all increase the half-life of $cPLA_2$ mRNA transcripts, indicating a role for post-transcriptional modulation of gene expression. The presence of three ATTTA motifs in the 3′ untranslated region (3′UTR) of the rat $cPLA_2$ cDNA is ascertained. Heterologous expression of chimeric constructs with different 3′UTRs ligated into the 3′ end of the luciferase coding region reveals that the presence of the $cPLA_2$ 3′UTR results in reduced luciferase activity compared with constructs without the $cPLA_2$ 3′UTR. Furthermore, the luciferase activity in the constructs with the $cPLA_2$ 3′UTR is increased in response to the same agonists which stabilize endogenous $cPLA_2$ mRNA. A negligible effect of these agonists on transcriptional control of $cPLA_2$ is evident using promoter–reporter constructs expressed in transient and stable transfectants. Taken together, these results indicate predominant post-transcriptional regulation of $cPLA_2$ mRNA levels.

## INTRODUCTION

The release of arachidonic acid (AA) from membrane phospholipids is believed to be the rate-limiting step in the elaboration of eicosanoids, which in turn are involved in a wide spectrum of biological processes, both physiological and pathological [1,2]. The release of AA from phospholipids is catalysed by phospholipases; amongst these, cytosolic phospholipase $A_2$ ($cPLA_2$) has been shown to be selective for AA at the *sn*-2 position and responsive to mitogenic and vasoactive agonists [3,4].

Previous studies have delineated a pathway for rapid activation of $cPLA_2$ by post-translational modification in response to epidermal growth factor (EGF), platelet-derived growth factor (PDGF), phorbol 12-myristate 13-acetate (PMA), cytokines and other agonists. Recent studies by ourselves [5] and others [6,7] have shown that many of these same agonists also increase steady-state $cPLA_2$ mRNa levels. Such increases could result either from an increase in transcription, a decrease in mRNA degradation, or both. In the current study, we have examined the relative contributions of transcriptional and post-transcriptional processes to the regulation of $cPLA_2$ gene expression in cultured rat glomerular mesangial cells. The latter are vascular smooth muscle-like cells of the glomerular microcirculation which have previously been used to study the effects of growth factors and mitogens on $cPLA_2$ mRNA levels and enzyme activity [5].

## MATERIALS AND METHODS

### Materials

Cell culture media, antibiotics, transferrin, insulin, selenium, glutamine, trace element mix and Albumax™ were obtained from Gibco BRL, Burlington, Ontario. Foetal bovine serum (FBS) was obtained from ICN Flow, Mississauga, Ontario. PMA and D-luciferin were obtained from Sigma, St. Louis, MO, U.S.A. PDGF was obtained from R&D Systems, Minneapolis, MN, U.S.A. EGF, actinomycin D, ATP and resocrufin-β-galactopyranoside were obtained from Boehringer Mannheim, Laval, Quebec.

### Plasmids

pTKβgal, a β-galactosidase expression vector containing the thymidine kinase promoter, was purchased from Pharmacia, Piscataway, NJ, U.S.A. Generous gifts of plasmids were received as follows: pA3LUC, a promoterless luciferase plasmid [8] from Dr. E. Chester Ridgway, University of Colorado; pFGB plasmid containing the human c-*fos* promoter [9] from Dr. N. Miyamoto, Ontario Cancer Institute, Toronto; murine $cPLA_2$ cDNA plasmid [3] from Dr. J. Knopf, Genetics Institute, Cambridge, MA, U.S.A.; pLUCHGH containing luciferase coding sequence with 3′ untranslated region (3′UTR) of human growth hormone gene (HGH), from Dr. D. Drucker, Banting and Best Diabetes Centre, Toronto.

The luciferase constructs p2487LUC, p1928LUC, p1003LUC and p185LUC have previously been described [10]. An additional construct, p470LUC, was generated by subcloning an Asp718-*Hin*dIII fragment of the rat $cPLA_2$ promoter [10] into pA3LUC. p*fos*LUC was generated by ligating a blunted *Cla*I-*Hin*dIII fragment of the human c-*fos* promoter into the *Sma*I site of pA3LUC.

### Cell culture

Homogeneous cultures of rat mesangial cells were obtained and characterized as previously described [11]. The experiments described were performed using mesangial cells between the 20th

Abbreviations used: $cPLA_2$, cytosolic phospholipase $A_2$; AA, arachidonic acid; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; PMA, phorbol 12-myristate 13-acetate; FBS, fetal bovine serum; 3′UTR, 3′ untranslated region; HGH, human growth hormone; DMEM, Dulbecco's modified Eagle medium; PBS, phosphate-buffered saline; CMV, cytomegalovirus; DDT, dithiothreitol; ARE, adenosine-uridine rich element; GAPD, glyceraldehyde phosphate dehydrogenase.

The sequence of the 3′UTR of the rat $cPLA_2$ cDNA has been deposited with Genbank and has accession number U08375.

* To whom correspondence should be addressed.

and 40th passages. Cells were propagated in Dulbecco's modified Eagle's medium (DMEM) containing 20% FBS with 50 U/ml penicillin and 50 μg/ml streptomycin in 5% $CO_2$ in air at 37 °C. Cells were rendered quiescent prior to each experiment by first rinsing with PBS then incubating for 24 h in serum-free medium consisting of DMEM:F12 containing 10 μg/ml transferrin, 10 nM sodium selenite, 1 × trace element mix, 1 mg/ml Albumax, 0.1 U/ml insulin and 1 mM glutamine as previously described [5]. *Balb*/c3T3 cells were obtained from American Type Culture Collection, Rockville, MD, U.S.A., and propagated in DMEM in 10% calf serum.

### Actinomycin D chase

Mesangial cell culture and serum starvation were performed as noted above and as previously described [5]. Following serum starvation, cells were incubated with each of the following agonists for 6 h: EGF 0.1 μM, PDGF 20 ng/ml, PMA 1 μg/ml or 20% FBS. Actinomycin D (6 μg/ml) was then added, and cells harvested at various time-points for RNA isolation. RNA isolation [12], Northern transfer and hybridization were carried out as previously described [5]. Experiments were performed in duplicate for each agonist.

### Isolation of 3′UTR of rat $cPLA_2$ cDNA

To determine if the ATTTA motifs present in murine and human $cPLA_2$ cDNA sequences also existed in the rat cDNA, a rat macrophage cDNA library (Clontech, Palo Alto, CA, U.S.A.) was screened with radiolabelled full-length murine $cPLA_2$ cDNA probes using standard protocols [13]. DNA was prepared from positively hybridizing bacteriophage clones, digested with *Eco*RI and subcloned into pSP72 (Promega, Madison, WI, U.S.A.) for dideoxy sequencing [14].

### 3′UTR chimeras

The effect of the 3′UTR of rat $cPLA_2$ on mRNA stability was assessed by measurement of luciferase activity following expression in rat mesangial cells of chimeric constructs comprising different 3′UTRs ligated to the 3′ end of luciferase. pLUCHGH consisted of a 600 bp PCR fragment containing the 3′UTR of the HGH gene cloned into the *Stu*I-*Sal*I sites of pLUC. The 3′UTR of the HGH gene was chosen because of the relative stability of its mRNA transcript [15]. pLUC consisted of 1 kb of the cytomegalovirus (CMV) promoter cloned into the *Bam*HI site of the pGEMLUC vector (Promega).

pDS1 was constructed by cloning a blunted 400 bp *Eco*RI fragment from the 3′UTR of rat $cPLA_2$ cDNA into the *Stu*I site of pLUCHGH. The orientation of the insert was confirmed by sequencing. pDS2 was constructed by cloning a 400 bp *Eco*RV-*Sal*I fragment from the 3′UTR of rat $cPLA_2$ cDNA into the *Stu*I-*Sal*I sites of pLUC. The plasmids were transiently transfected into rat mesangial cells in triplicate as described below. Transfected cells were serum-starved and then incubated with agonist or vehicle. Luciferase assays of cell lysates were performed as described below.

### Transient transfections

The ability of the agonists EGF, PMA, PDGF and serum to induce an increase in $cPLA_2$ steady-state mRNA in rat mesangial cells has been reported previously [5]. Transient transfection of rat mesangial cells and *Balb*/c3T3 fibroblasts was performed using the $CaPO_4$ co-precipitation method with the modification of Chen and Okayama [16]. Cells were plated at a density of $5 \times 10^5$ cells per 10 cm diameter dish 1 day before transfection and returned to a 5% $CO_2$ incubator at 37 °C. All plasmids used in transfections were purified by two spins through CsCl gradients on a tabletop ultracentrifuge (Beckman, Optima TL™) at a speed of 100000 rev./min. The concentration of DNA was measured by absorbance at 260 nm on a Beckman DU-62 spectrophotometer. Equimolar quantities of each reporter plasmid were used to transfect the cells, using 12.5 μg per dish for the plasmid of highest molecular weight in each set of transfections. Co-transfection with 7.5 μg of pTKβgal was performed to allow normalization of luciferase activity with an efficiency of transfection as assessed by β-galactosidase activity. pSP72 vector was added where necessary, to make up 20 μg of total DNA in each transfection. Sham transfections used 20 μg of pSP72 plasmid. Transfections were done in triplicate, after which cells were placed in an incubator with 3% $CO_2$ at 35 °C. Sixteen hours later, cells were rinsed twice with PBS, placed in fresh medium and then returned to the 5% $CO_2$ incubator at 37 °C. For inducibility experiments, cells were placed in serum-free medium after rinsing. As a positive control in initial experiments, cells were transfected with p*fos*LUC, containing the c-*fos* promoter linked to pA3LUC. The luciferase activity of cells transfected with this plasmid was consistently increased 10-fold upon serum stimulation.

### Stable transfections

Stable transfectants were generated by co-transfection of promoter–reporter constructs along with pSV2Neo, followed by selection of cells in G418 (Gibco BRL, Burlington, Ontario). Initially, mesangial cells were used but transfected cells failed to survive in G418 medium, so stable transfectants were generated in *Balb*/c3T3 fibroblasts instead.

Cells were plated at a density of $5 \times 10^5$ cells per dish, and duplicate dishes were transfected with 18 μg of reporter plasmid and 2 μg of pSV2Neo, using $CaPO_4$ co-precipitation as described for transient transfections. The cells were placed in DMEM 48 h after transfection with 600 μg/ml G418. Colonies of G418-resistant cells could be distinguished after 14–16 days. Multiple resistant colonies were pooled to ensure a population of cells with a heterogeneity of integration sites. Stable transfectants at 70% confluence were serum-starved for 24 h before stimulation with agonists of interest. Cells were harvested for luciferase and protein assays after 12 h of incubation with agonist.

### Luciferase assay

For assay of luciferase activity, each dish of transfected cells was rinsed three times with ice-cold PBS, then treated with 0.3 ml of ice-cold lysis buffer [100 mM $KH_2PO_4$, pH 7.8; 1 mM dithiothreitol (DTT)]. The cells were then lysed by three freeze–thaw cycles. After the last thaw, cell lysates were centrifuged briefly and the supernatants collected. Samples containing equivalent amounts of protein were added to 0.3 ml of reaction buffer (15 mM $MgSO_4$, 15 mM potassium phosphate, 1 mM DTT, 2 mM ATP) and assayed in a Monolight 2010 luminometer (Analytical Luminescence Laboratory, San Diego, CA, U.S.A.). Each luciferase measurement was done in duplicate.

### Protein measurements and β-galactosidase assay

Protein content of cell extracts was determined by the Bradford procedure [17] using Bio-Rad protein assay reagents (Bio-Rad, Richmond, CA, U.S.A.). Equivalent amounts of cell extracts were incubated with β-galactosidase assay buffer [50 mM Tris/HCl (pH 7.5)/10 mM $MgCl_2$/100 mM NaCl] and reso-

crufin-$\beta$-galactopyranoside substrate at 30 °C until a colour change from orange to red was noted [18]. Absorbance was then measured at 572 nm using sham-transfected cells as blank.

### Data analysis

Transfection data shown are representative of at least triplicate experiments except in the case of inducibility experiments with 3′UTR constructs which were performed in duplicate for each agonist. Summary results are presented as means ± S.E.M.

## RESULTS

To determine the effect of agonists on $cPLA_2$ mRNA stability, rat mesangial cells were pretreated with agonists or vehicle before incubation with actinomycin D, which is a transcription inhibitor that acts by intercalating into nucleic acids. First we established that after 24 h of serum starvation, the level of $cPLA_2$ mRNA was in a steady state for at least the subsequent 6 hours in the absence of actinomycin D or agonist addition (results not shown). In contrast, the addition of actinomycin D resulted in a steady decrease in $cPLA_2$ mRNA with an apparent half-life of 3 h. Pretreatment of cells with PMA stabilized the $cPLA_2$ transcript, such that there was no significant decrease in message even at 5 h after addition of actinomycin D (Figure 1). Similar results were seen with PDGF (Figure 2), EGF and 20% FBS (results not shown). Although there was some inter-experiment variation in the absolute amount of $cPLA_2$ mRNA present at time zero following serum starvation, the percentage of original RNA remaining at each time-point was comparable in duplicate experiments performed for each agonist.

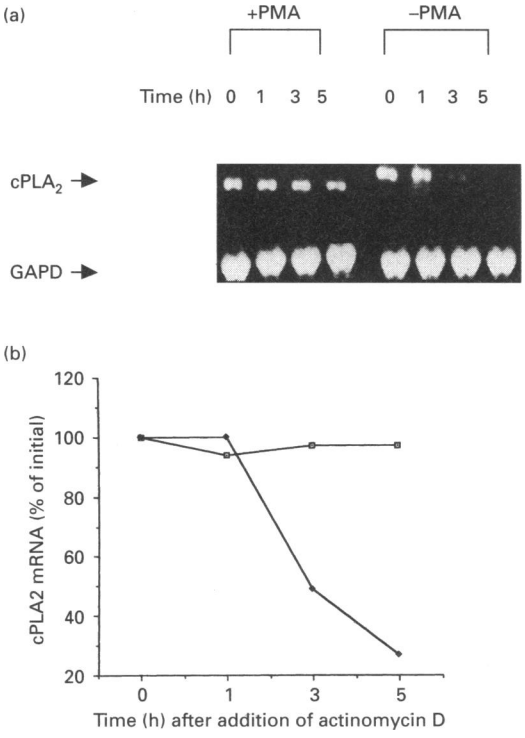


**Figure 1 Effect of PMA on $cPLA_2$ mRNA stability**

Rat mesangial cells were serum-starved for 24 h and then incubated with either PMA (1 $\mu$g/ml) or vehicle for 6 h. Cells were harvested for RNA isolation at various intervals after addition of actinomycin D (6 $\mu$g/ml). (**a**) Northern-blot analysis of actinomycin D chase of cells pretreated with PMA or vehicle. (**b**) Graphical representation of the data from (**a**). RNA abundance was measured by densitometry of bands on Northern blot and normalized for corresponding abundance of mRNA for glyceraldehyde phosphate dehydrogenase (GAPD). mRNA abundance in relative densitometric units at time zero was set at 100%. □ Denotes pretreatment with PMA and ◆ denotes pretreatment with vehicle. The experiment was performed in duplicate, with comparable results.

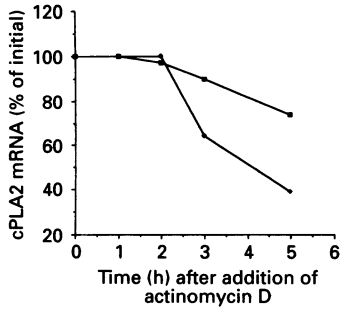


**Figure 2 Effect of PDGF on $cPLA_2$ mRNA stability**

Experimental conditions were the same as in Figure 1 except that cells were stimulated, □, with 20 ng/ml PDGF or ◆, with vehicle for 6 h before treatment with actinomycin. The experiment was performed in duplicate, with comparable results.

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Rat     ATTTAAACAT TCCTCACATT TTTACCTGAG GCAATTTTTA
Mouse   TTTTAAACAT TCCTCACATT TTTTACCTGT ACTTTTTATA
Human   TCCTGATACA AATGTAGGGA TATATACTGT ATTTTTAAAC

Rat     TATATATAAA AATATCTTTC CTTTTATAAA TATTTAATAG
Mouse   TAAATATGAC ATGTCTTTTC TTTTGAAAAT ATTTAATAGT
Human   ATTTCTCACC AACTTTCTTA TGTGTGTTCT TTTTAAAAAT

Rat     TTTAACTCAG TAAAAAAAGC TTCCCATTGT GTGTGAATGT
Mouse   TTAACTCAGT AAAGGAGACT TCCCATTGTG TGTGAATGTT
Human   TTTTTTTCTT TTAAAATATT TAACAGTTCA ATCTCAATAA

Rat     TATTCTGAAC TAGATTTGTT CATGCCATGT TACAACACTA
Mouse   ATTCTGAACT AGATTTGTTC ATGCCATGTT ACAACACTAT
Human   GACCTCGCAT TATGTATGAA TGTTATTCAC TGACTAGATT

Rat     TTTTTATTTA AATGTTCATA TCT
Mouse   TTTTATTTAA ATGTTTATATTTA
Human   TATTCATACC ATGAGACAAC ACTATTTTTATTTA
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**Figure 3 Alignment of ARE-containing sequences in the 3′UTR of the $cPLA_2$ gene in rat, mouse and human species**

The ATTTA motifs are denoted in bold and underlined. The sequence of the 3′UTR of the rat $cPLA_2$ cDNA has been deposited with Genbank and has accession number U08375.

Since AU-rich elements (ARE) which potentially confer message instability have been identified in the $cPLA_2$ gene in the human and mouse species, we sought to ascertain if the rat gene contained similar motifs. Screening of a rat macrophage cDNA library identified three positive bacteriophage clones from which DNA was purified and analysed. After digestion with *Eco*RI, the largest insert was subcloned into pSP72 and mapped. This was found to contain two positively hybridizing fragments of about 2.4 kb and 400 bp respectively. Both fragments were partially sequenced using SP6 and T7 primers. The 2.4 kb fragment was found to correspond to the 5′ untranslated and coding regions of $cPLA_2$, while the 400 bp fragment contained the 3′UTR, including the poly(A) tail. The complete sequence of the 400 bp fragment was obtained and was found to contain ATTTA motifs, and comparison with the murine sequence revealed that the position of two of these was conserved (Figure 3).

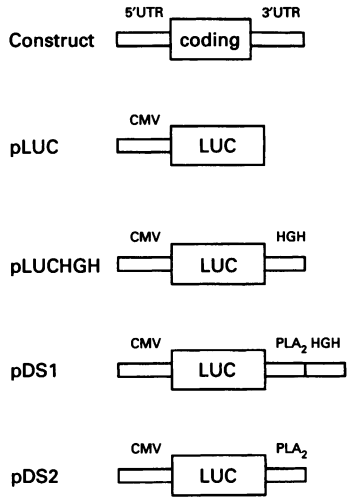


**Figure 4 3'UTR chimeric constructs**

pLUC consisted of 1 kb of the CMV promoter cloned in the *Bam*HI site of pGEMLUC vector. pLUCHGH consisted of a 600 bp polymerase chain reaction fragment containing the 3'UTR of the HGH gene cloned into the *Stu*I-*Sal*I sites of pLUC. pDS1 was constructed by cloning a blunted 400 bp *Eco*RI fragment from the 3'UTR of rat $cPLA_2$ cDNA into the *Stu*I site of pLUCHGH. pDS2 was constructed by cloning a 400 pb *Eco*RV-*Sal*I fragment from the 3'UTR of rat $cPLA_2$ cDNA into the *Stu*I-*Sal*I sites of pLUC.

The effect of the ARE-containing 3'UTR on $cPLA_2$ mRNA stability was assessed by comparison of luciferase activities of chimeric constructs with different 3'UTRs instead (Figure 4). Insertion of the 3'UTR of rat $cPLA_2$ cDNA into the luciferase expression plasmid, pLUCHGH, led to a decrease in luciferase activity (Table 1). A similar effect of the 3'UTR of rat $cPLA_2$ cDNA to reduce luciferase activity was observed, when the 3'UTR of rat $cPLA_2$ cDNA replaced the 3'UTR of pLUCHGH (Table 1). Furthermore, the presence of the 3'UTR of rat $cPLA_2$ consistently conferred a 2-fold inducibility of luciferase activity in response to the agonists PDGF, PMA, EGF and 20% serum. In contrast, no such induction was observed in plasmids lacking the 3'UTR of rat $cPLA_2$ (Table 1).

We have previously mapped the start site of transcription for the rat $cPLA_2$ gene and isolated its promoter [10]. In order to assess the potential additional contribution of transcriptional regulation of $cPLA_2$ gene expression, we examined the effects of those agonists which increase $cPLA_2$ mRNA levels on luciferase activity of rat mesangial cells transiently transfected with $cPLA_2$ promoter–luciferase constructs. Using different $cPLA_2$ promoter–luciferase constructs containing varying lengths and regions of 5'-flanking DNA, only minimal induction of luciferase activity was observed in response to agonist stimulation in most cases (Table 2). The maximum induction observed was a 2-fold increase in luciferase activity in response to PDGF and PMA in mesangial cells transiently transfected with the p1003LUC construct, which contains nucleotides −1003 to +40 of the 5'-flanking region of the $cPLA_2$ gene. Promoter inducibility was also examined using both transient and stable transfectants generated in *Balb*/c3T3 cells. A similar pattern of minimal or no induction of luciferase activity was elicited in response to the test agonists (results not shown). The site of transcription initiation of the transgene was mapped using 5'RACE with the aid of a 5'RACE kit (Gibco BRL) in the *Balb*/c3T3 cells stably transfected with p470LUC, and was found to correspond to that which we previously reported for the endogenous $cPLA_2$ gene ([10], results not shown).

## DISCUSSION

We have reported that EGF, PDGF, FBS and PMA increase steady-state levels of $cPLA_2$ mRNA in mesangial cells in culture [5]. Pretreatment of cells with cycloheximide was associated with

**Table 1 Relative luciferase activity and inducibility of 3'UTR chimeric constructs in transiently transfected rat mesangial cells**

| Construct | Relative luciferase activity (%) | Fold inducibility* | | | |
|---|---|---|---|---|---|
| | | PDGF (20 ng/ml) | PMA (1 μg/ml) | EGF (0.1 μM) | 20% FBS |
| pCMVLUC | 10±0.2 | 1.00 | 1.40 | 1.31 | 1.00 |
| pHGHLUC | 100±0.3 | 1.00 | 1.41 | 1.00 | 1.10 |
| pDS1 | 64±0.4 | 1.75 | 3.33 | 1.84 | 1.72 |
| pDS2 | 30±0.4 | 1.81 | 2.89 | 1.90 | 2.00 |

*Data represent the mean of two experiments with each agonist.

**Table 2 Effects of agonists on luciferase activities of transiently transfected rat mesangial cells**

| Construct | Ratio of stimulated:unstimulated luciferase activity | | | |
|---|---|---|---|---|
| | PMA (1 μg/ml) | PDGF (20 ng/ml) | EGF (0.1 μM) | 20% FBS |
| pA3LUC (Vector) | 1.1±0.0 | 1.0±0.0 | 1.0±0.1 | 1.1±0.1 |
| p200LUC | 1.1±0.1 | 1.2±0.1 | 1.0±0.1 | 1.0±0.0 |
| p470LUC | 1.8±00 | 1.0±0.1 | 1.4±0.1 | 1.1±0.1 |
| p1003LUC | 2.5±0.1 | 2.0±0.1 | 1.0±0.0 | 1.1±0.1 |
| p1928LUC | 2.1±0.1 | 1.9±0.1 | 1.1±0.1 | 1.1±0.1 |
| p2487LUC | 2.7±0.1 | 1.8±0.0 | 1.1±0.0 | 1.3±0.1 |
| pTKLUC | 1.1±0.1 | 1.3±0.1 | 1.1±0.0 | 1.0±0.0 |

superinduction of gene expression, suggesting possible involvement of a labile mRNA-degrading protein in the regulation of transcript abundance [5]. Indeed, in one study it was shown that macrophage colony stimulating factor increased the half-life of $cPLA_2$ mRNA in human monocytes [6]. In order to discern the potential contribution of regulation at the level of mRNA stability, the current study examined the decay of $cPLA_2$ mRNA levels following actinomycin D treatment in mesangial cells.

Modulation of gene expression by alteration of mRNA stability is a major control point that provides a powerful means for controlling gene expression [19]. One sequence that has been studied extensively in the regulation of mRNA decay is the ARE. The destabilizing influence of the ARE has been demonstrated elegantly by Shaw and Kamen [20] in chimeric constructs of the $\beta$-globin gene, whose normally stable mRNA was made labile by the insertion of an ARE from the 3′UTR of granulocyte-monocyte colony stimulating factor. However, only a subset of endogenous ARE-containing mRNAs have been observed to be stabilized following incubation with various agonists [19,20].

We observed that the rat $cPLA_2$ cDNA has three ATTTA motifs distributed over a 180 bp region in the 3′UTR. Accordingly, the potential functional significance of the putative ARE-containing 3′UTR of the rat $cPLA_2$ cDNA was tested in a series of experiments using chimeric constructs in which the 3′UTR of the rat $cPLA_2$ cDNA was added to, or replaced, the 3′UTR of the HGH gene in a luciferase expression vector, pLUCHGH. A luciferase expression vector was used to allow the indirect assessment of RNA stability by luciferase assays of rat mesangial cells transiently transfected with these constructs. Insertion of the 3′UTR of the $cPLA_2$ cDNA immediately 5′ to the HGH 3′UTR led to a decrease of approx. 40% in luciferase activity in serum-starved mesangial cells compared with cells transfected with pHGHLUC. Replacement of the 3′UTR of the HGH with the 3′UTR of the $cPLA_2$ cDNA resulted in an even greater decrease in luciferase activity to only 30% compared with pLUCHGH. Luciferase activity in cells transfected with the constructs containing the 3′UTR of $cPLA_2$ was consistently increased by treatment with those agonists which stabilized endogenous mRNA half-life and increased steady-state transcript levels. In contrast, cells transfected with pLUCHGH showed no significant increase in luciferase activity following stimulation with EGF, PDGF, serum, or PMA. These findings suggest that the ARE-containing 3′UTR of the $cPLA_2$ gene does indeed contribute to the destabilization of its mRNA transcript and this destabilizing effect is overcome, at least in part, by treatment with the same agonists that have been shown to increase steady-state $cPLA_2$ mRNA. Further validation of this interpretation requires formal studies of mRNA decay using cells stably transfected with chimeric constructs of the 3′UTR of $cPLA_2$ linked to a gene with a stable mRNA.

Given the central role of $cPLA_2$ in cell signalling and the involvement of AA metabolites in cell growth responses [1,2], it is perhaps not surprising that there exist post-transcriptional mechanisms for the regulation of $cPLA_2$ mRNA stability, and at the same time post-translational mechanisms [21,22] for regulating the activity of the protein. It is of interest to note that the gene encoding cyclo-oxygenase-2 immediately downstream in the AA–eicosanoid cascade is also reported to display post-transcriptional regulation at the level of mRNA stability [23].

The foregoing results do not rule out an additional component of regulation of $cPLA_2$ gene expression at the transcriptional level. Transcriptional regulation of genes by agonists can be assessed by nuclear run-on assays. However, in agreement with reports from other laboratories [6], a sufficient signal for nascent $cPLA_2$ nuclear mRNA in run-on assays could not readily be detected, even when differential expression of transcripts for various other genes such as $\beta$-actin and c-*fos* could be demonstrated (results not shown). This is presumed to reflect the low abundance of the $cPLA_2$ transcript. As an alternative approach to analysis of transcriptional regulation, we studied the inducibility of $cPLA_2$ promoter activity in response to agonists in cells transfected with $cPLA_2$ promoter–luciferase reporter constructs.

Transient transfection studies using both rat mesangial and *Balb*/c3T3 cell lines demonstrated little or no increase in luciferase activity for most of the promoter constructs tested in response to the agonists which had been shown in parallel to increase $cPLA_2$ steady-state mRNA under the same incubation conditions. Under these same experimental conditions, transfection with a c-*fos* promoter–luciferase plasmid resulted in a 10-fold increase in luciferase activity with serum stimulation (results not shown), consistent with published data [24]. Stable transfectants generated in *Balb*/c3T3 mouse fibroblasts also showed minimal or no induction in response to agonists. These findings suggest either that these agonists do not influence the transcriptional rate of the $cPLA_2$ gene, or alternatively that elements mediating transcriptional responses to these agonists reside outside the promoter regions studied. However, a prominent contribution at the level of transcription rate is not likely, since agonist-induced changes in $cPLA_2$ mRNA levels could be accounted for to a great extent by effects at the level of mRNA stability.

Our results demonstrate that $cPLA_2$ gene expression in cultured rat mesangial cells is regulated post-transcriptionally by PMA, PDGF, EGF and serum, which have previously been shown to increase steady-state mRNA levels of $cPLA_2$ in the same cells. Studies using chimeric 3′UTR constructs suggest that the ARE-containing 3′UTR of the rat $cPLA_2$ gene is responsible, at least in part, for instability of the transcript. Further analysis of the functional domains of the 3′UTR of the $cPLA_2$ gene may help to delineate the molecular and cellular mechanisms modulating the stability of $cPLA_2$ mRNA transcripts.

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